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# INTERFERENCE OPTICAL SCANNER

Final Report

March 1974 to June 1975

by

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November, 1975

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DEPARTMENT OF THE ARMY
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| ollaston prism | Amplitude comparator | Periplan ocular | Birefringement lenses |

ABSTRACT (Continue on reverse side if necessary and identify by block number)

A 2-milliwatt He-Ne laser and beam-forming optics were used in a new ght source. Solid state silicon photodiodes replaced the former photomulti-lier tube. Two units, a two-channel amplitude comparator (to use the outputs rom the interference channel and the normal channel to determine whether articles scanned are transparent or opaque, rejecting the latter) and a Pulse eight/Pulse Width discriminator (which sorts particles according to size and pacity) were fabricated. Normarski prisms were abandoned as part of the etector system and some success with Wollaston prisms was had where

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lateral separation of beams occurred. Wollaston prisms produced a 1° divergence or recombining angle. A third, 0.6° Wollaston prism was added later to restore parallelism to the emerging light. The optical system of an operational interference optical scanner was studied. Optical interference can be used on a PACT system to render transparent organisms readily visible. More work is needed to perfect techniques and optical subassemblies for this last application.

# PREFACE

The work described in this report was authorized by Contract DAAA15-74-C-0175, Interference Optical Scanner. It was carried out from March 1974 to June 1975.

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# INTERFERENCE OPTICAL SCANNER

## 1.0 INTRODUCTION

The purpose of this program has been to develop a new technology for use in the PACT (Pattern Acquisition and Correlation Technique) system. This new technology is intended to permit the elimination of the troublesome staining process currently employed, by using optical interference techniques to detect transparent organisms. Under this program, a basic guidline has been to retain the basic PACT hardware and build on a foundation of existing, proven methods.

By way of background, the function of this PACT optical system is to sample the ambient air continuously by depositing airborne particles onto a clear plastic tape and subjecting these particles to a protein stain and, subsequently, scanning them with a microscopic flying spot comparable in size to individual bacteria, etc., which are to be observed. The flying spot is viewed by two photomultiplier tubes (PMT's), one through a red filter and one through a green filter. By observing the relative amplitudes of these two video channels, distinction between stained organisms and opaque particles is made by the electronics. An opaque particle is of no interest, while a stained particle suggests the presence of bacteria. This system is highly sensitive, as the presence of a single organism can be detected in a clean laboratory environment.

Under recent developmental contracts, this system has yielded good performance. However, it suffers from several disadvantages which result from the use of the stain.

- False Alarm Rate and Alarm Sensitivity

  Failure mode analysis of the stain/destain concept has shown that even partial failure of the destain step inherently increases the false alarm rate and impairs the ability to detect low level aerosols.

  All of the aerosol particles on the tape are stained and are thus sensitized to detection and represent a potential false alarm source. Consequently, failure of the destain step to remove all the stain from the non-biological materials increases the false alarm source. Partial failure increases the average background level and consequently reduces detection capability.

- Time to Alarm

  The stain and destain apparatus occupies several inches of tape length between the impaction point and the scanner, so that an additional delay in the time to alarm is encountered between the time an air sample is picked up and the time it is observed. Obviously, elimination of 1 to 2 minutes from the alarm time can significantly blunt the effectiveness of an actual attack.

- Mobility

  Elimination of the liquid stain and destain makes it possible to develop a system that can operate in a moving and vibrating environment and is insensitive to position. This should greatly simplify operation and set up in the field.

- Logistics and Service

  Elimination of the stain and destain requirements remove the need to handle reagent chemicals in the supply system as well as the need for regular servicing of the equipment under field operating conditions.

- Reliability and Maintenance

  The stain and destain apparatus adds to the complexity of the system, thereby degrading overall system reliability and imposing the requirements of additional adjustment and maintenance procedures.

The drawbacks enumerated above can be circumvented by eliminatin the stain/destain concept and adopting a different technique than dye coloring as a means to separate biological particles from opaque inorganic particles. Optical interference techniques, which are in wide use in microscopy, offer just such an alternative. In interference, the light is separated into two distinct components prior to illuminating the specimen One component is both focused on the specimen and made to pass through it while the other is not subjected to both of these conditions. In the image plane, the two components recombine with constructive and/or destructive interference due to phase difference, resulting from optical path difference. This interference renders a completely transparent object highly visible.

The interference approach provides a means of distinguishing between different transparent materials on the basis of size (in the direction of light travel) and index of refraction. Hence proteinaceous materials can be distinguished easily from other transparent materials. Since the optical path difference between the image forming beam and the reference beam is determined by the product of these parameters, the amplitude of the video signal (a function of the resultant phase difference between the two beams) also will be related to this product. Thus, it may be possible to distinguish between different transparent particles by using pulse height/width sorting as is being done currently on the PACT system.

Opaque particles can be rejected using a second channel with common mode pulse rejection similar to the logic used in the current PACT system. By not recombining the reference beam with the interference beam, the transparent particles would be rendered either invisible or greatly diminished in amplitude, thereby making possible a second video channel which only is able to see semitransparent or opaque particles. Thus, it is very simple to build an electronic device which rejects any video signal which is "seen" in both video channels.

## 2.0 SUMMARY

As stated in the Introduction, the purpose of this program has been the development of new technology for the PACT scanning microscope through the application of optical interference techniques. The principal goals of this program have been to develop and implement an optical interference technique using a GFE Mark VI PACT System. In addition to this implementation of this optical hardware, appropriate signal processing electronics for isolating and sorting challenge signals were designed and fabricated.

The specific tasks of this program were specified as follows:

a. Design the optical system.

b. Conduct optical breadboard tests.

c. Design two electronic subsystems.

d. Fabricate and test these electronic systems.

e. Modify the GFE system to accommodate the new optical configuration.

f. Integrate the optical and electronic subsystems.

g. Conduct a final checkout of the integrated system.

For simplicity, the tasks a, b, and e were incorporated into a single task, since the initial optical design experimentation could be carried out efficiently using the Mark VI optical assembly. This work began with the removal of the zirconium light source and the staining apparatus. A two milliwatt helium-neon laser was installed together with the necessary beam forming optics, as the new light source. In addition, the old photomultiplier tube (PMT) was removed and replaced with solid-state silicon photodiodes. This modification was made possible because of the high light level produced by the laser, and it substantially simplifies the design of future systems because the diodes do not require a high voltage power supply as does the PMT, and also the diodes are physically much more compact. To accommodate the diodes, simple electronics were required to couple their outputs to the existing electronics. Additional electronics, consisting of two units for signal processing, were fabricated. The first of these is the Two-Channel Amplitude Comparator whose purpose is to utilize the outputs from the Interference Channel and the normal Channel to determine if the particles scanned are transparent or opaque and consequently to reject the latter. The second unit is a Pulse Height/ Pulse Width Discriminator which sorts particles on the basis of size and opacity as is being done currently on the conventional systems using stain.

The initial optical configuration examined utilized a pair of Normarski prisms (a modified form of the Wollaston prism). A pair of these prisms was installed in the system in order to produce a separation of the beam into two beams and subsequently to recombine them. However, since these prisms were designed for use with a different optical system (which is also not compatible with the PACT configuration), inadequate beam separation resulted and proper operation of the system was not obtained.

Subsequently, two approaches utilizing longitudinal rather than lateral beam separation were investigated. One technique involved the use of thin birefringent calcite plates cemented to the outermost element of both the condensing and image-forming objectives. This approach, however, was found to introduce serious optical aberrations and thus was discarded as impractical. This method may be feasible, however, if specially designed condensers and objectives were employed. A second approach, utilizing a pair of birefringent lenses, of a type sometimes referred to as "curved Rochon prisms" was implemented. The particular type of prism appears as a positive lens to light vibrating in one plane, but as a flat window to light vibrating in a plane normal to the first. This approach however, did not permit simultaneously a uniform magnification of the two images and a recombining of the two images in the same focal plane. Moreover when the images were recombined, the resultant different magnification was accompanied by different convergence rates on the two beams, so that a series of interference rings was produced, instead of a uniform extinction over a sizable area.

Finally, an approach utilizing a pair of conventional Wollaston prism was investigated, which was similar in concept to the modified Nomarski approach mentioned earlier. This approach produces a lateral separation of the beams. The Wollaston prisms employed produce a 1° divergence (or recombining) angle which is adequate for this application. Later a third, 0.6° Wollaston prism was added to the system in order to restore parallelism to the emerging light. This approach produced a broad null covering a substantial portion of the field of view and gave interference contrast of higher amplitude while scanning unstained Bg. However, the video waveform was complex in structure and did not show a clear resemblance of the subject being scanned. However, due to the encouraging results obtained using this method, efforts toward further investigation and refinement of this approach are recommended. A two fold goal of such a continuation in the development of this particular approach would be (1) to increase the width of the null to cover essentially the entire scan line, and (2) to resolve a representation of the original structure of the material being scanned.

Substantial progress has been made during this program in research and development toward an operational interference optical scanner. The most critical part of this program has been the effort toward perfecting the optical technique to be employed in such a system. Unstained Bg organisms have been prominently visible using Wollaston and Nomarski prism approaches, and these techniques commend themselves for future work toward perfecting them.

The present program has demonstrated successfully that optical interference can be used on a PACT system to render transparent organisms readily visible. Future work should be directed toward refining the technique used, design and fabrication of a more suitable optical subassembly for this application and overall system integration and testing.

# 3.0 THEORETICAL BACKGROUND

The drawbacks of conventional PACT systems list in Section 1.0 can be circumvented by eliminating the stain/detain concept and adopting a different technique than dye color to separate biological particles from opaque inorganic particles. These disadvantages are summarized in the introduction.

In order to circumvent the disadvantages which result from the use of staining, optical interference techniques offer an attractive alternative, since they render organic transparent material visible in the absence of stain. In order to provide a more complete understanding of the work done on this program, we shall now examine the underlying theory of interference contrast microscopy as it relates to work done on this contract.

## 3.1 Basic Principles of Phase Contrast Microscopy

### 3.1.1 Theory of Operation

The underlying principles of interference contrast microscopy is the use of two light beams in the image forming process of the microscope. One of these beams is focused upon the subject to be observed, in the usual fashion, while the other beam is employed as a phase reference in the plane of the final image. The first beam which we shall call the imaging beam must travel a different optical distance when passing through transparent material, due to the different index of refraction of the transparent material. Thus, the phase of this beam as seen in the image plane will be shifted from that which would have been observed in the absence of the transparent particle. If the second beam, termed the reference beam has been so adjusted in phase that, in the absence of an object in the light path it is 180° out of phase with the imaging beam and of equal amplitude, a dark field will result because of the cancellation of the two beams. Thus, any perturbation in either phase or amplitude of the imaging beam will result in an incomplete cancellation of the two beams in the focal plane causing the object which perturbed the reference beam to appear as a bright object against the dark background. It is evident, therefore, from this explanation that even a perfectly transparent object will appear distinctly against the dark background, as long as its index of refraction differs sufficiently from the ambient medium and its thickness is sufficiently great to produce a significant phase shift in the reference beam. This condition is easily realizable with bacteria as long as air rather than immersion oil is used as the ambient medium. Unfortunately the indices of refraction of immersion oils often may be sufficiently close to the indices of refraction of many transparent organisms to fail to provide a large enough phase shift to render the organisms visible. For this reason and also to simplify the system both in terms of simplicity of operation in the field and in terms of reliability and maintenance--free operation, it is recommended that the use of oil immersion be eliminated on future versions of this system. It is not anticipated that the decreased optical resolution resulting from such a modification should have a serious impact on system performance.

Figure 1 is an optical schematic of the Interference Optical Scanner as implemented in the Mark VI optical subassembly. Here a 2-milliwatt helium-neon laser has been installed in place of the zirconium arc lamp. The light from the laser passes through the original folding mirror, galvanometer and Leitz periplan ocular, to a birefringent beam-splitting prism (1° Wollaston prism shown). This prism separates the light into two very slightly displaced beams (not distinguished in Figure 1, but shown schematically in Figures 4 and 5 where they are drawn separately. The two beams are orthogonally polarized. The beams pass through a folding mirror (also used in original Mark VI), through condenser and objective through another original folding mirror, to a second 1° Wollaston birefringent prism which recombines the two beams, followed by a 0.6° Wollaston phasing prism which restores parallelism to the two recombined beams. A quarter wave plate restores plane polarization to the recombined beams (when no wave plate restores plane polarization to the recombined beams (when no specimens are present on the tape) and the polarized analyzer cancels out the resultant so that a low brightness level is obtained when no specimen is present. A phase perturbation in one of the beams upsets this darkened condition so that more light passes through the analyzer, making even perfectly transparent particles highly visible. A second reference or "normal" channel (not shown) can be used in parallel which simply cancels out the reference beam, so that only opaque or dark objects are visible, providing a reference channel for the logic to faciltate rejection of opaque particles. The configuration shown in Figure 1 is a 3° Wollaston lateral displacement system which has yielded encouraging results. It is discussed in Section 3 below and illustrated in Figures 5 and 18 (photograph) with oscilloscope photographs of video data from carbon and Bg in Figures 19, 20, and 21.

### 3.1.2 Types of Interference Contrast Systems

As mentioned above, the interference contrast microscope utilizes two beams of light in its operation which we have termed the imaging beam and the reference beam. The imaging beam is focused on the specimen in the same manner as with a conventional microscope; however the reference beam is not in focus at the same identical point. Interference contrast microscopes do differ, however, in how the reference beam is handled by the optics. Although other variations are possible, two basic approaches are used conventionally in terms of the focal position of the reference beam. One approach displaces the reference beam laterally from the imaging beam so that it comes to a focus in the same plane as the imaging beam but in a different location. Such systems may be called lateral displacement systems. The other approach displaces the focal point longitudinally along its axis of revolution, so that the focal point of this beam is outside of the specimen plane, while the central axes of the two beams are coincident. Such systems may be termed longitudinal displacement systems. Obviously, a hybrid system in which the focus of the reference beam is displaced both laterally and longitudinally is also a possible third

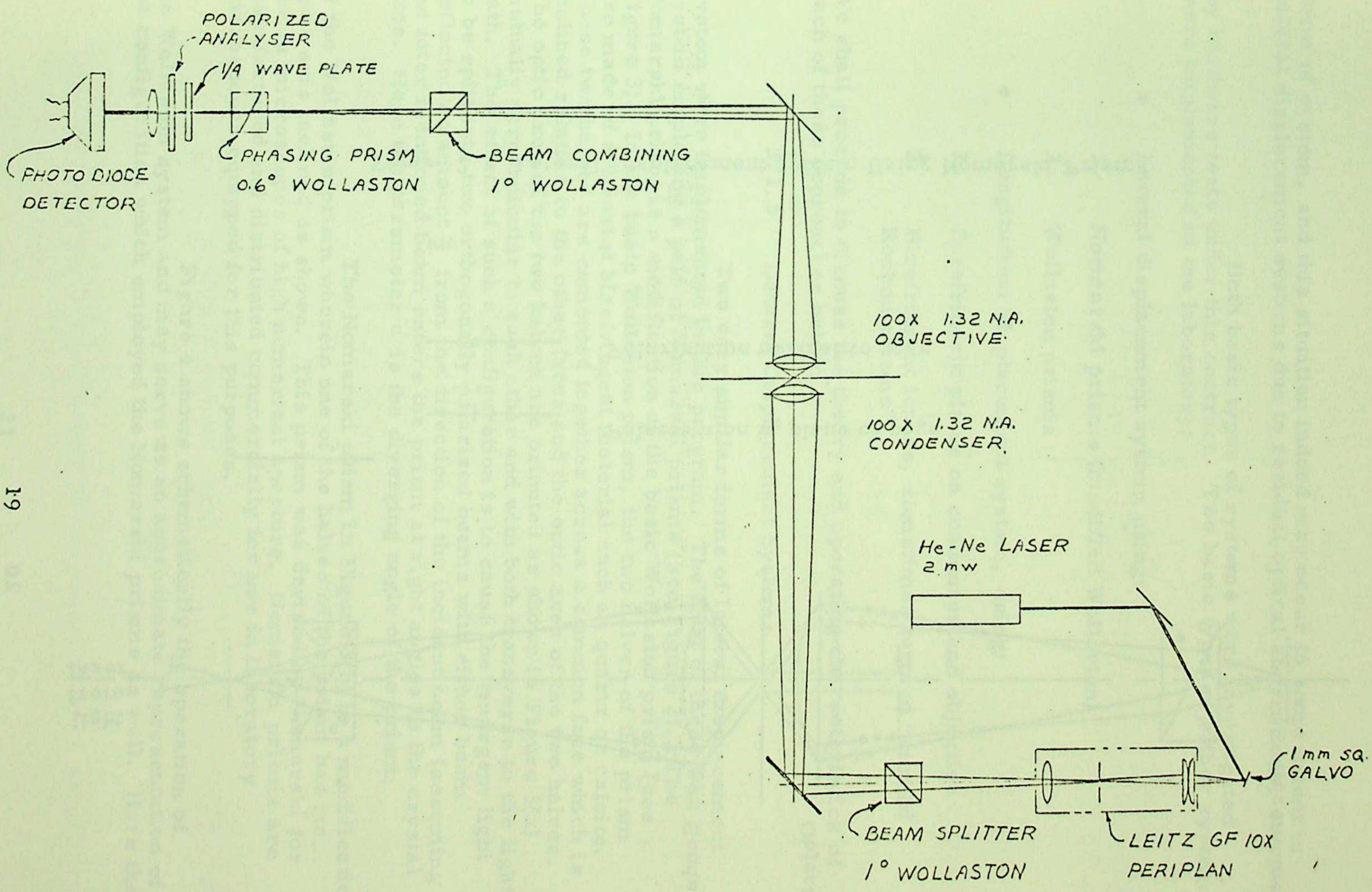


Figure 1 Optical Schematic of Interference Optical Scanner

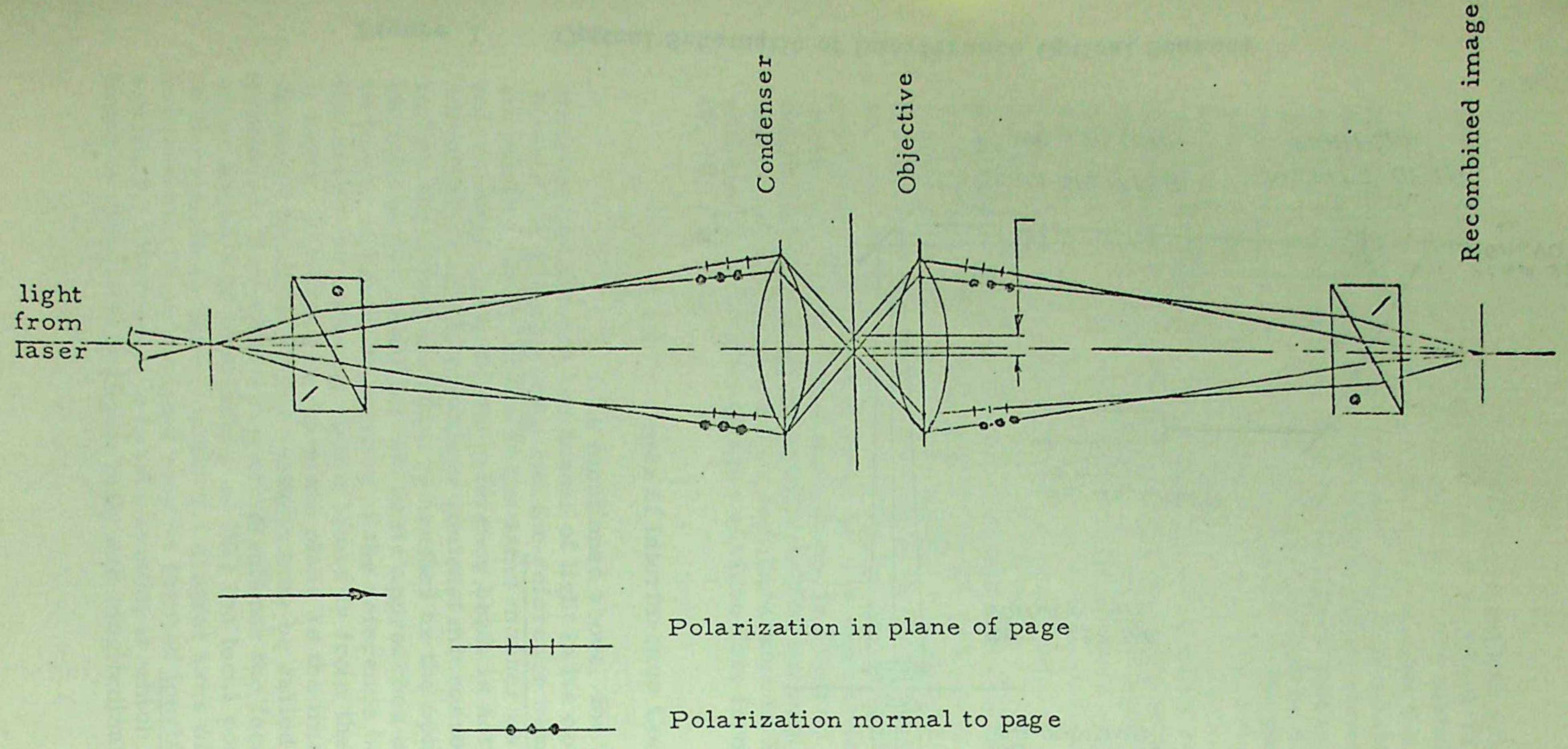


Figure 2 Lateral Displacement System Using Nomarski Prisms

form of system, and this situation indeed may occur to some extent in lateral displacement systems due to residual optical aberrations, etc.

Both basic types of systems were investigated by hardware tests under this contract. Two basic types of either system were implemented in the laboratory:

- Lateral displacement system using:
    1. Normarski prisms (modified Wollaston)
    2. Wollaston prisms
- Longitudinal displacement system, using:
    1. Birefringent plates on condenser and objective
    2. Birefringent lenses, sometimes termed "curved Rochon prisms"

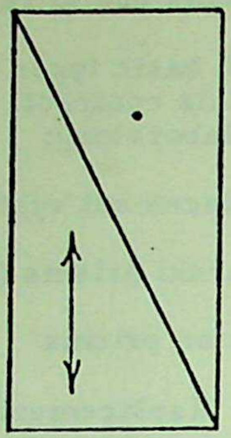
We shall procede to discuss the theory and operating characteristics of each of these approaches below.

### 3.1.3 Lateral Displacement Systems

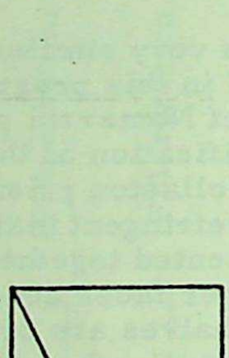
Two very similar forms of lateral displacement system were implemented in this program. The first of these was a system employing a pair of Nomarski prisms (see Figure 2). The Nomarski prism is a modification of the basic Wollaston prism (see Figure 3). In the basic Wollaston prism, the two halves of the prism are made of a uniaxial birefringent material such as quartz or calcite. These two halves are cemented together across a common face which is inclined relative to the other faces and the optic axes of the two halves. The optic axes of the two halves are oriented as shown in Figure 3(a) mutually perpendicular to each other and with both transverse to the light path. The effect of such a configuration is to cause the emergent light to be split into two orthogonally polarized beams with either beam deflected an amount $\frac{\alpha}{2}$ from the direction of the incident beam (assuming the later mentioned beam enters the prism at right angles to the crystal axes. Here the parameter $\alpha$ is the diverging angle of the prism.

The Nomarski prism in Figure 3(b) is a modification of the Wollaston prism wherein one of the halves of the prism has its optic axis inclined as shown. This prism was devised by Nomarski for use in microscopes of high numerical aperture. Nomarski prisms are manufactured and distributed commercially for use in laboratory microscopes equipped for this purposes.

Figure 4 shows schematically the operation of the Wollaston system and may serve as an approximate representation of the configuration which employed the Nomarski prisms as well. Here the



(a) Wollaston Prism



(b) Nomarski Prism

Figure 3 Comparison of Wollaston and Nomarski Prisms

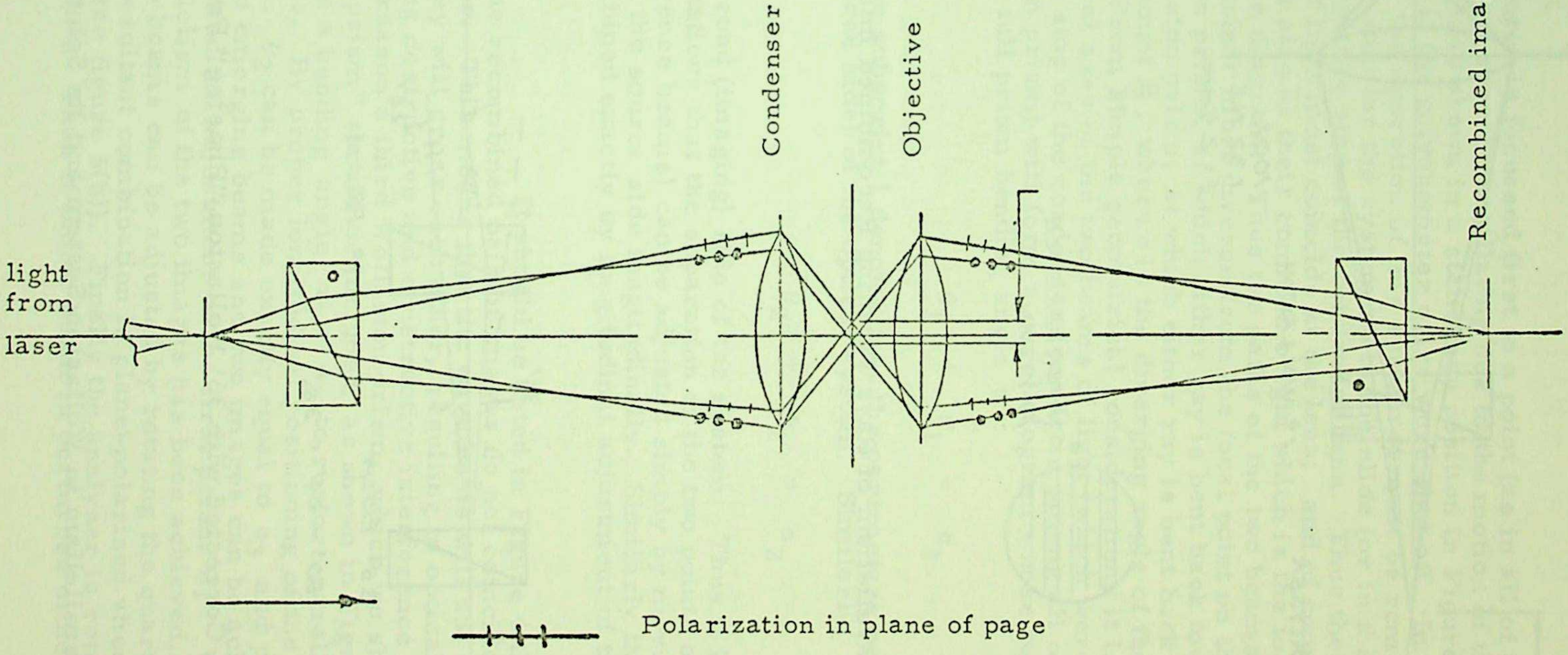
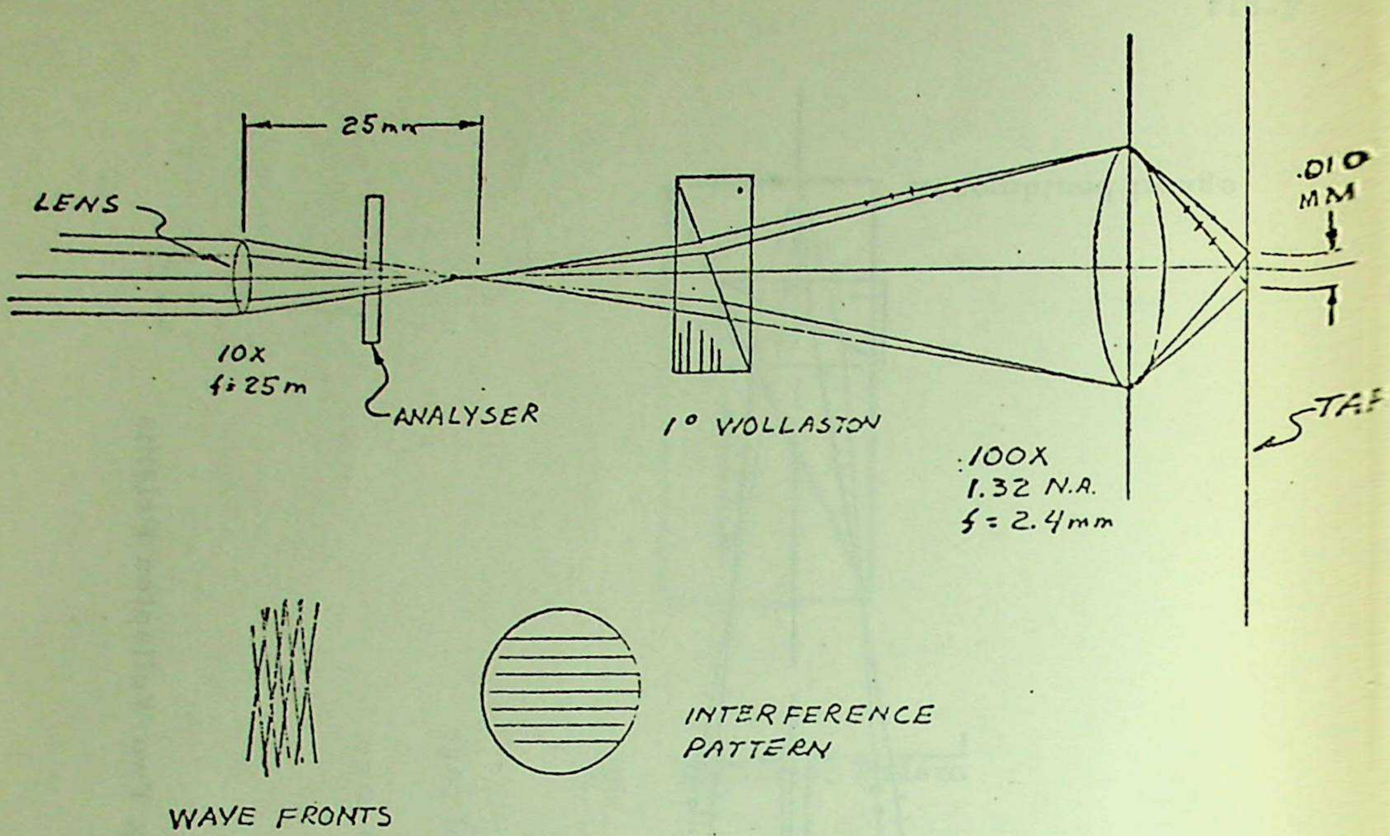
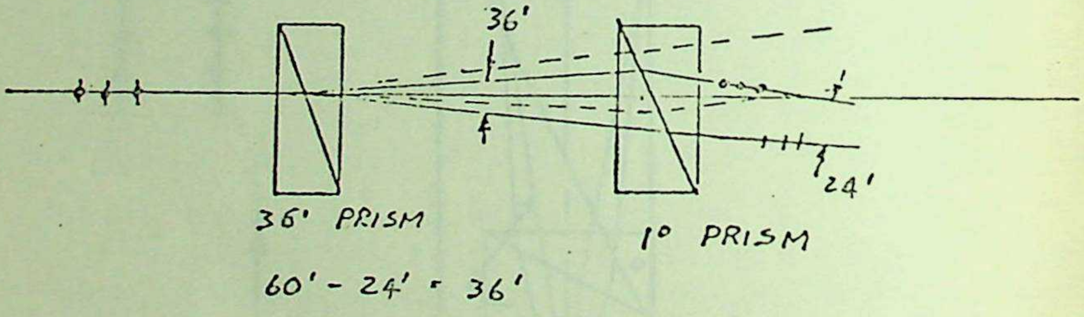


Figure 4 Lateral Displacement System Using Two Wollaston Prisms



(a) Effect of Non-Parallelism of Rays Forming Recombined Image



(b) Non-Parallelism Corrected with 36' Wollaston "Phasing" Prism

Figure 5 Non-Parallelism in Wollaston System and Its Solution

laser source is focussed first to a point (as in all of the systems). This point is, of course, oscillating due to the motion of the galvanometer, although it is shown in a stationary position in Figure 4, as would be the case if the galvanometer drive were shut off. In order to understand properly the operation of this system it must be remembered that the aperture stop for the system up to the slide (or in this case, plastic tape) is the aperture stop of the condenser lens. Thus the boundaries of the two beams of light must coincide at the lens; and of course, they must intersect also at their common origin which is the focal point at the far left of the diagram. Thus the paths of the two beams of light selected by the condenser must diverge from the focal point on the left toward the Wollaston prism, at which either ray is bent back toward the center by an Wollaston prism, at which either ray is bent back toward the center by an amount $\frac{\alpha}{2}$, where $\alpha$ is the diverging angle of the prism, as discussed above. From simple geometrical considerations it is easy to show that the central axes of the two beams of light (which were selected by the aperture stop of the condenser lens from among all of the light bent by the Wollaston prism) will form a parallelogram whose two acute angles add up to the full prism bending angle $\alpha$ :

$$\theta_1 + \theta_1 = \alpha_1 \tag{1}$$

Where the subscript 1 denotes that the parameters pertain to the first side (source side) of the optical system. Similarly, it follows that:

$$\theta_2 + \theta_2 = \alpha_2 \tag{2}$$

on the second (imaging) side of the system. Thus it is easy to see from these equations that the separation of the two points of light (foci of imaging and reference beams) can be adjusted simply by moving the Wollaston prism on the source side longitudinally. Similarly the two images can be recombined exactly by longitudinal adjustment of the second Wollaston prism.

It should be noted in Figure 4 that the central axes of the recombined pair of images do not coincide, but rather cross each other. This means that the wavefronts will not coincide, but rather they will cross each other, resulting in optical fringes of alternating destructive and constructive interference (see Figure 5(a)). For this reason a third Wollaston prism, which we shall refer to as a "phasing prism" should be included, as shown in Figures 5(b). This prism must have a bending angle $\alpha_3 = \theta_2$ to restore parallelism between the two beams. By proper longitudinal positioning of the second Wollaston prism $W_1$, $\theta_2$ can be made exactly equal to $\alpha_3$ and precise coincidence of the two emerging beams and two images can be achieved by careful final longitudinal adjustment of the first prism $W_1$. When coincidence and parallelism of the two images has been achieved, the relative phase of the two beams can be adjusted by rotating the quarter wave plate so that the resultant combination is plane-polarized when no specimens are present (see figure 5(b)). Finally the analyzer is rotated to a null for the resultant. Thus if either beam is perturbed either in amplitude or phase, the resultant recombined image will be changed in polarization, producing a brightening against a dark background as stated earlier.

A second lateral displacement system which is very similar to the one described in the above paragraphs is the configuration utilizing Nomarski prisms. The Nomarski prism is a modified Wollaston in which the element which does not have its optic axis parallel to the cleavage plane between the two elements, the optic axis of that element is no longer parallel to the entrance and exit faces of the prism. Instead, this axis is inclined toward the cleavage plane (see Figure 3(b)). These prisms are manufactured commercially for use in microscopes and therefore are readily available.

The first system examined experiementally in this program was this system using two of these Nomarski prisms. This system functions in essentially the same way as does the basic Wollaston system just described. Therefore the above discussion regarding the basic two-prism Wollaston system and Figure 4 suffice for this variant of the Wollaston configuration.

Early tests, using the Nomarski prisms, were made with immersion oil and no definitive interference results were observed with unstained Bg. Later tests without oil gave very encouraging results, and this approach holds great promise for future development.

### 3.1.4 Longitudinal Displacement Systems

An alternative approach to displacing the imaging and reference beams laterally is to displace their foci longitudinally in the specimen plane. In such an arrangement, the central axes of the two beams are coincident, and the imaging beam is in focus on the specimen while the reference beam is out of focus at the specimen. Such an approach has one distinct advantage in that the reference beam is out of focus in the plane of the slide or clear plastic tape, and thus the reference beam is affected to a much lesser degree by the specimens whic may cross its path, than would be the case with a focused beam.

One conceptually simple method of achieving longitudinal displacement of the two foci is shown in Figure 6. Here a thin plate of birefringent calcite is cemented to the face of the outer element of both the condenser and objective lenses with the optic axes both perpendicular to the central axis of the microscope optical train and also perpendicular to each other. The incoming light from the laser source is polarized at 45° to the two optic axes. When the focused beam passes out of the condenser through the first of these two plates, the two vector components of the light undergo different amounts of refraction due to the birefringence. After leaving the plate the two cones of light are displaced longitudinally. If an identical birefringent plate is placed beyond the two foci, with its optic axis at right angles to the optic axis of the first plate, the two beams will emerge recombined. In practice this second plate can be cemented to the outer element of the objective.

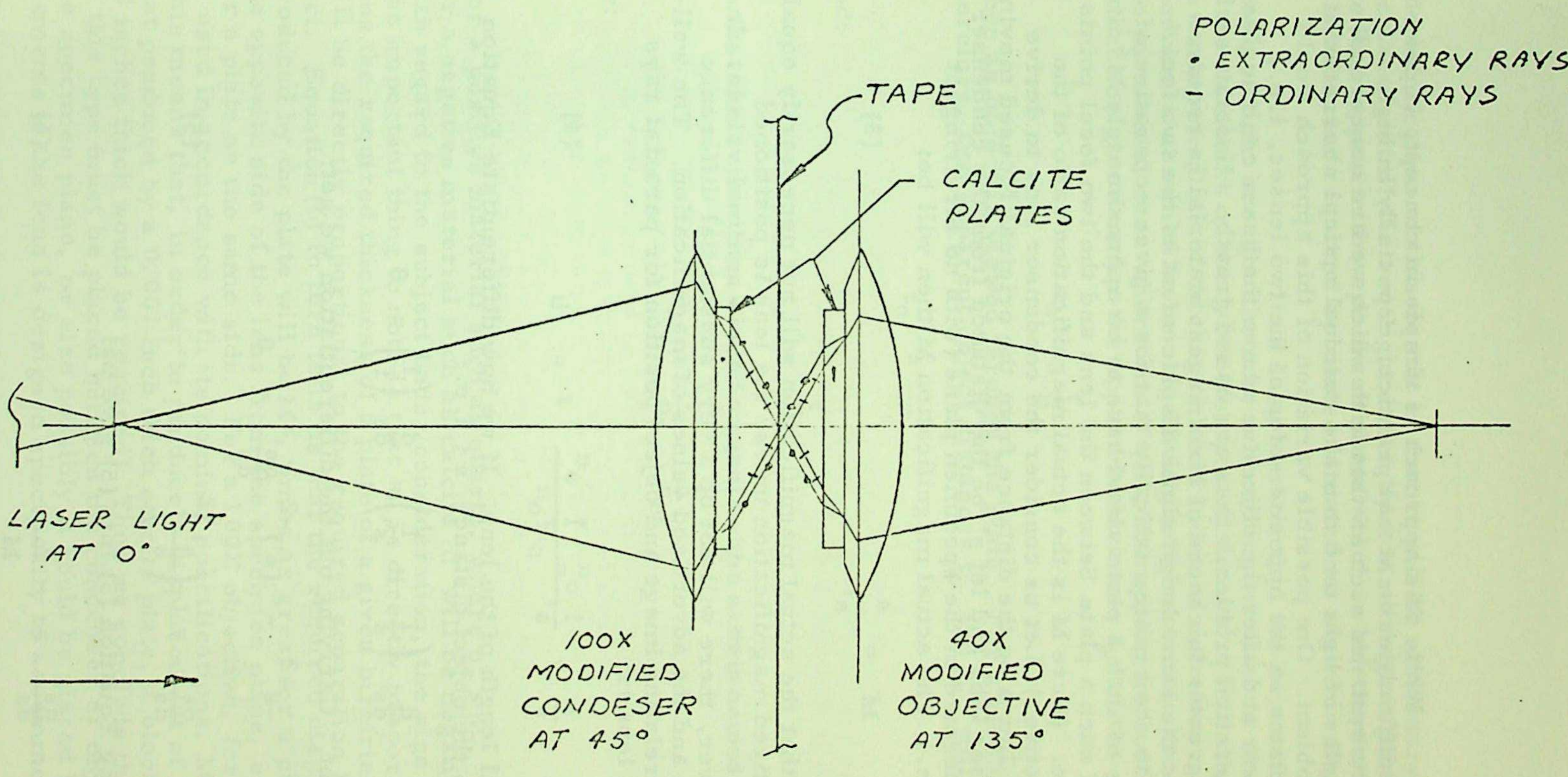


Figure 6 Longitudinal Displacement System Using Calcite Plates

While this approach is simple in concept, it does introduce serious difficulties in actual practice, especially in high numerical aperture systems such as that with which we are concerned. The steep cone angles of light tend to make residual optical aberrations a very serious problem. One possible variation of this approach would be to use calcite plates on the opposite sides of the two lenses, i. e., before the condenser and after the objective where the beam cone angles are small and aberration problems thus are eased greatly. Unfortunately, however, a much greater thickness of birefringent material is required in order to achieve the same longitudinal displacement of the two foci. Indeed, it is easy to show mathematically that for a given separation of foci, the thickness of such a plate would have to be approximately $M^2$ times as great as that of such a plate between the lens and the two focal points as described above. (Here M is the actual magnification ratio of the condenser or objective.) Let us consider the condenser lens to derive this relationship. Let s be the distance from the original focused moving spot to the condenser lens and let s' be the distance from the condenser lens to the image formed in the specimen plane (with no birefringent plate in the system.) The actual magnification M then will be:

$$M = \frac{s}{s'} \qquad (3)$$

It should be noted that the actual magnification will not necessarily equal the nominal advertized magnification unless the lens is positioned relative to the source so that s and s' assume their nominal values. In most cases, however, there will not be a very substantial difference between the actual and the advertized values of magnification. The well-known expression relating image and object position for paraxial rays and a thin positive lens is:

$$\frac{1}{s} + \frac{1}{s'} = \frac{1}{f} \qquad (4)$$

where f is the focal length of the lens. If we now differentiate Equation (3) with respect to the object distance s , i. e. :

$$\frac{d}{ds}\left(\frac{1}{s} + \frac{1}{s'}\right) = \frac{d}{ds}\left(\frac{1}{f}\right) = 0$$

since f is a constant. Carrying out the differentiation we get:

$$-s^{-2} - (s')^{-2}\,\frac{ds'}{ds} = 0$$

so that:

$$\frac{ds'}{ds} = -\left(\frac{s'}{s}\right)^2 \qquad (5)$$

Finally, substituting Equation (3) into (5) give us:

$$\frac{ds'}{ds} = -\frac{1}{M^2} \qquad (6)$$

The implication of this result is that it will be necessary to introduce a displacement of the two light cones by a birefringent plate on the side of the lens opposite the specimen plane which is $M^2$ times as large as the displacement required by plates situated as shown in Figure 6.

It is easy to show that the separation between two cones of light brought about by a birefringent plate is directly proportional to the thickness of the later (for paraxial rays). Initially let us consider an isotropic plate of thickness t and index of refration n. The surrounding medium is assumed to be air, having a refractive index of unity. Assuming the para-axial case where $\sin \theta \approx \theta$, the application of Snell's law of refraction gives us for the apparent thickness $t_a$ of the plate:

$$t_a = \frac{t}{n}$$

when an object is viewed through the plate. For a birefringent plate therefore, two apparent object distances $t_o$ and $t_e$ will be seen so that:

$$t_o = \frac{t}{n_o}$$

and:

$$t_e = \frac{t}{n_e}$$

so that the separation which is equal to the difference $d = t_o - t_e$ will be

$$d = \frac{t}{n_o} - \frac{t}{n_e}$$

or:

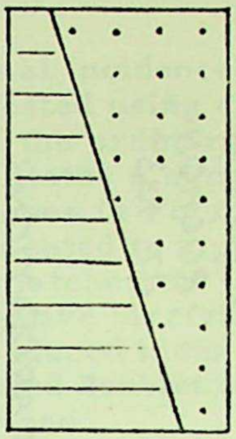
$$d = t \frac{n_e - n_o}{n_o n_e} \qquad (7)$$

For a positive material such as quartz, where $n_e > n_o$, d will be positve; for a negative material such as calcite d will be negative. Of course, with regard to the subject under consideration, the sign is of no concern. The important thing to note is that d is directly proportional to t, thus the required thickness of a plate of a given birefringent substance will be directly proportional to the required separation between the two foci. Equation (6) in turn tells us that the required displacement produced by the plate will be $M^2$ times as great for a plate situated on the opposite side of the lens from the specimen plane, as would be required for a plate on the same side. For a 100X objective, for example, if it is used in accordance with its nominal specifications, $M^2$ will be $100^2 = 10^4$. This means that, in order to produce a displacement of foci the same as that produced by a 0.001 inch thick calcite plate, a block of calcite 10 inches thick would be required! Thus we conclude that the use of plates of this type must be placed between the condenser or objective lens and the specimen plane, or else possibly it could be placed between the lens elements if the lens is designed specifically to accommodate it.

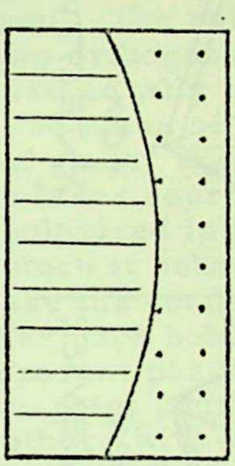
Thus far we have confined our mathematical calculations to the case of paraxial rays, i.e. to the case where the system has been stopped down to an infinitesimal aperture. Unfortunately these expressions generally do not remain valid for light rays passing through the outer zones of the system and which are bent through steep angles. Also we have not taken into consideration the case of objects and images which are away from the central axis of the system, i.e., away from the center of the field-of-view. When these factors are considered, it is found that the introduction of plates between the lenses and the specimen plane, where, as we have seen, they must be placed in order to have a significant effect, enormous amounts of aberration, at least of spherical aberration will be introduced. This approach was implemented during the program and it was found that this aberration problem precludes its use in the system under consideration. However, it may be noted that this approach, or a variation using the birefringent plates between lens elements, should prove satisfactory if the objective lenses are specially designed to work with the plates, thereby bringing residual aberrations back down to an acceptable level.

An alternative approach for introducing longitudinal displacement of the two foci is the use of a pair of birefringent lenses. A birefringent lens is one which contains at least one birefringent element so that its focal length will be different for the two beams of light passing through it, because of the distinction between their planes of polarization. One such lens may be regarded as a derivative of the Rochon prism with a curved interface between the two elements ( see Figure 7). As in the conventional Rochon prism, one element has its optic axis parallel to the light path, while the other element has its optic axis traverse to the light path, as is true of both elements in the Wollaston prism. The net result is that light passing through the first mentioned element experiences the same index of refraction regardless of polarization (assuming axial propagation). In the latter element, since the axis is transverse to the light path, a splitting of the light into two orthogonally polarized components will take place at the boundary, unless the incident beam is normal to the boundary surface. If the two elements are made of the same material, one ray will cross the boundary undeviate while the other will be deflected, with the magnitude and direction of this deflection being determined by the indicence angle at the boundary and als the ratio of the two indices of refraction. Clearly if this boundary is give a curvature, the prism will be changed into a birefringent lens which will be either converging (positive) or diverging (negative) depending upon the choice of birefringent material and which crystal orientation faces the convex side of the curve. It is also evident that while one ray (extraordinary ray) is affected as in a conventional lens, the other ray (ordinary ray) is undiverted as if passing through a plane window.

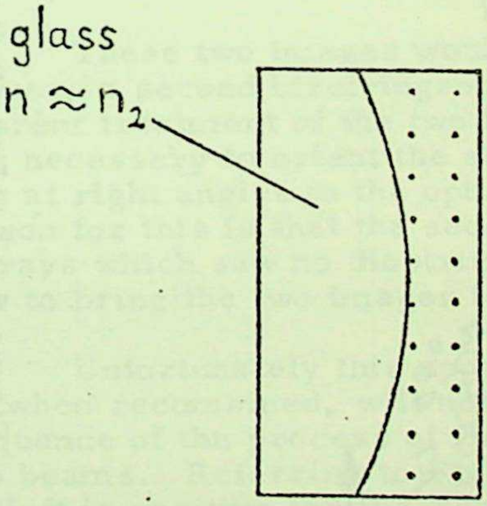
Since the resultant birefringent component has dioptric power, it now is practical to locate it on the side of the condenser or objective away from the specimen plane, thereby reducing aberrations to an acceptable level. Figure 8 depicts such a system using a pair of these birefringent lenses. Since the original concept one element has the optic axis parallel to the light path, both polarization experience the same



Rochon Prism



Curved "Rochon" Lens



Curved "Rochon" Lens With
One Glass Element

Figure 7 Rochon Prism and Birefringent Lens Derivations

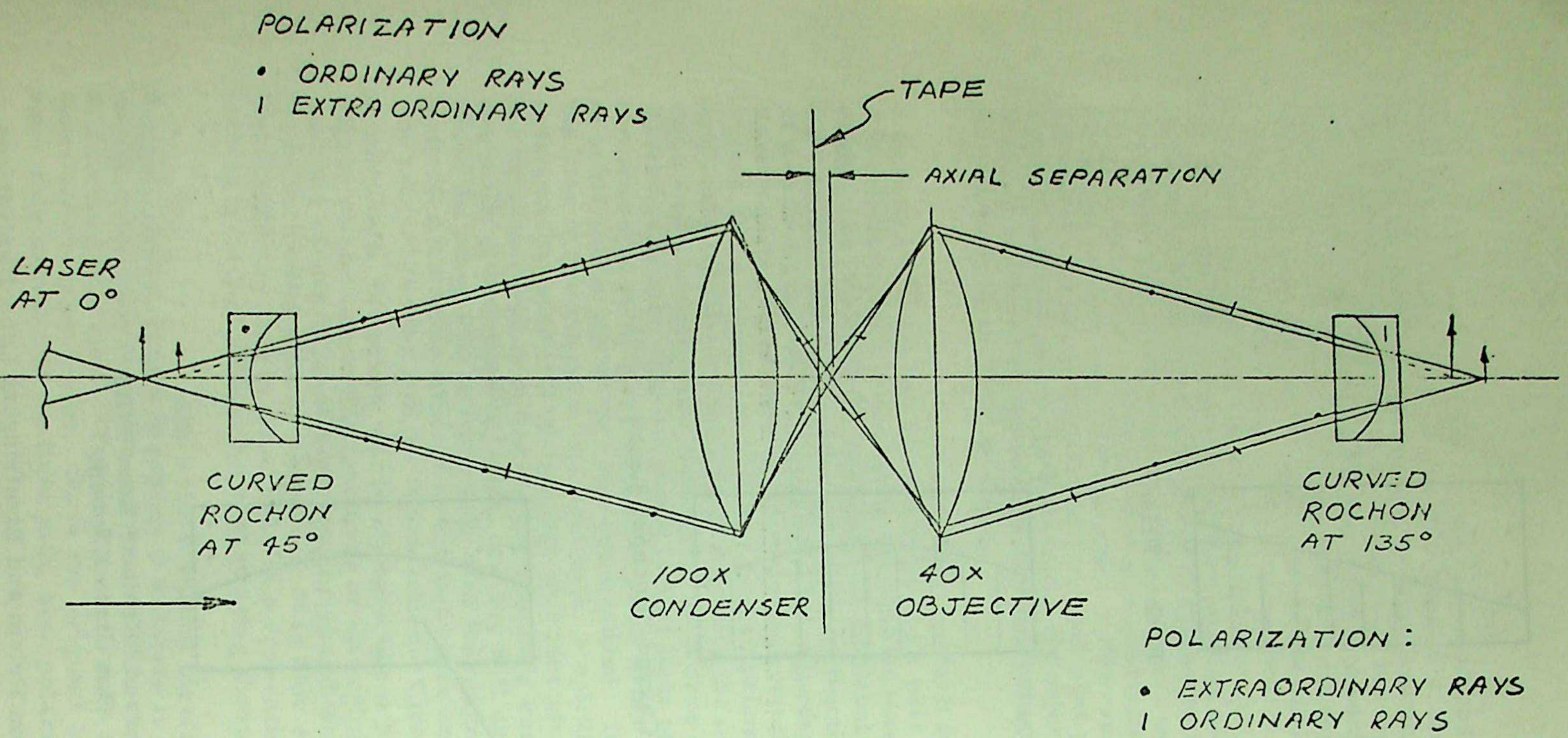


Figure 8 Longitudinal Displacement System Using Birefringent Lenses

index of refraction (assuming normal incidence). For this reason the birefringent lens also can be fabricated using a piece of glass having the same index of refraction as that of the ordinary ray in the birefrigent material. Such a substitution facilitates a simplification of the manufacture of the birefringent lenses. The lenses in Figure 8 contain a plano-concave element of birefringent calcite cemented to a plano-convex element of glass whose index of refraction closely matches the index for the ordinary ray in calcite. Since calcite has a negative birefringence, i.e. $n_e < n_o$, the extraordinary ray experiences a reduced index of refraction in the plano-concave element so that the cemented doublet appears as a positive, i.e. converging lens to light thus polarized.

The configuration portrayed in Figure 8 was implemented in the Mark VI system with two identical birefringent lenses located as shown. The two lenses were situated with the plano-convex glass elements facing the tape and the optic axes of the two calcite elements perpendicular to each other as shown. The polarization of the laser is at a 45° angle with respect to these two orthogonal orientations. Thus the light is split by the first lens into two equally intense beams. The beam polarized in a direction normal to the plane of the paper passes through the lens as if through a flat piece of glass, thereby causing only a slight shift in the apparent position of the laser source, as seen by the condenser lens. The component of the light polarized in the plane of the paper, however, "sees" a positive lens, since it behaves as the extraordinary ray and is bent accordingly. In this case the condenser lens "sees" a virtual source which is considerably further back behind the birefringent lens (to the left in Figure 8) than the apparent position of the first source. Thus the condenser lens is reimaging two point sources which are polarized orthogonally with respect to each other, with one of them (the one polarized in the plane of the paper) located to the left of the other (the one polarized normal to the plane of the paper). Two longitudinally displaced images are thus formed by the condenser lens as shown, in accordance with the basic rules of geometrical optics (see Figure 8).

These two images would then be reimaged by the objective lens; however, a second birefringent lens is placed as shown so that, due to the different treatment of the two beams, the two images are superimposed. It is necessary to orient the second birefringent lens so that the optic axis is at right angles to the optic axis of the first (see Figure 8). The reason for this is that the second lens must behave as a positive lens to the rays which saw no dioptric power at the first lens and vice versa, in order to bring the two images to a common focus.

Unfortunately this approach suffers a drawback in that the two images when recombined, will not be of equal size. This situation is a consequence of the process of first separating and then recombining the two beams. Referring to Figure 8 we note that the birefringent lens on the left is positive for the light which is polarized normal to the paper, and as a consequence the condenser "sees" a virtual image of the focussed laser beam (and scan) which is also magnified as a result of the action of this positive lens. On the right side of Figure 8 where the beams are reimaged, this beam (polarized normal to the page) is essentially unaffected by the second birefringent lens, while the other beam (polarized in the plane of the page) which was essentially unaffected by the lens on the right, undergoes a positive, converging action at the lens on the left. This causes the final image of the second beam to be minified as a result of the action of the second lens while the first lens magnifies the image of the first beam. Thus, the process of recombining the two images actually compounds rather than compensates the difference in magnification introduced by separating them. In addition, the difference in magnification causes the spherical wavefronts forming the two beams to interfere in such a manner as to produce an interference pattern consisting of a series of concentric rings (see Figure 9(a)).

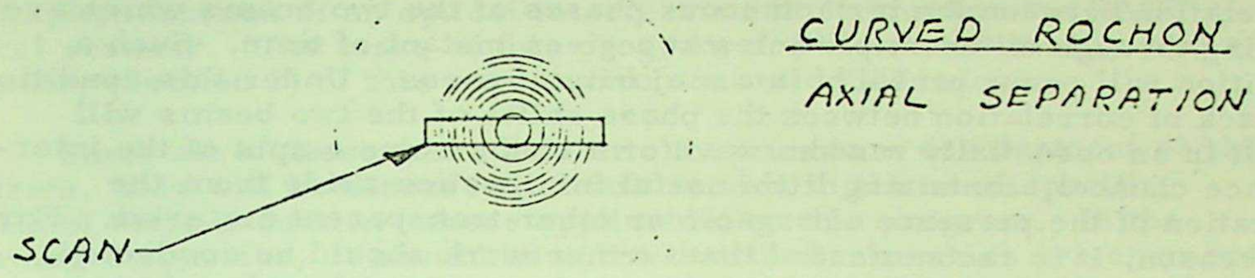
This approach also was implemented on this progra and it was found that this ring pattern just described persisted despite attempts to achieve a broad null. Modifications of the technique were tried, such as the addition of simple negative lenses to the birefringent lenses in order to produce combinations having equal positive and negative dioptric powers for the two beams; however, the interference pattern problem persisted.

### 3.1.5 Evaluation of Potential Merit of Approaches

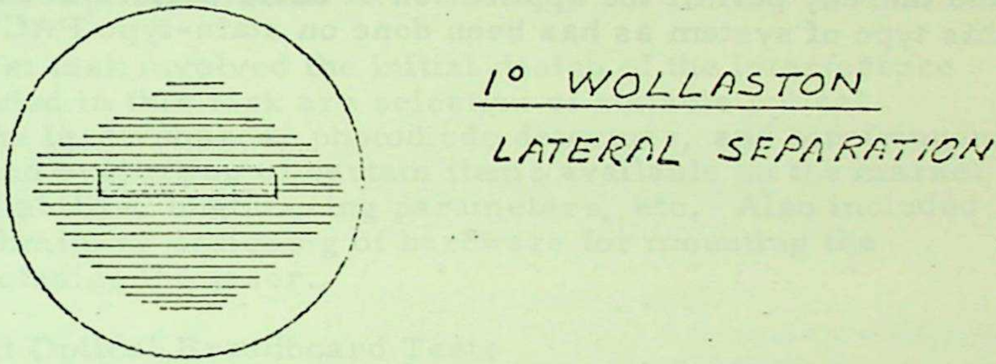
The methods just described have been examined experimentally in the course of the contract and consideration given to the feasibility of each on the basis of the tests conducted in the laboratory and also on the basis of theoretical considerations regarding the feasibility of circumventing various problems encountered in order to obtain acceptable interference patterns which do not suffer from the occurrence of multiple fringes and which permit a good sensitivity to the presence of transparent, organism-sized particles. Also the approach must not introduce objectionable amounts of optical aberrations.

On the basis of these considerations, the lateral displacement approaches appear much more promising than the longitudinal displacement techniques. With regard to the longitudinal approaches tried, the first method described (using thin birefringent plates) exhibited severe aberrations which cannot be eliminated without the use of specially designed lenses (i.e. the objective and/or condenser for example) and it appears questionable as to how well even special compensating optics can correct this problem. In this case of the use of the birefringent lenses, the problem of eliminating, or at least reducing to an acceptable level the circular fringes does not appear to be practical. This is further complicated by the problem of image size matching to which it may be related. However, since it is the phase relationship between the two beams (and hence images) which is important, it is not necessary to have uniform magnification of the two images although it is entirely possible that this condition will have to be satisfied in order to achieve a uniform phase cancellation over a wide area.

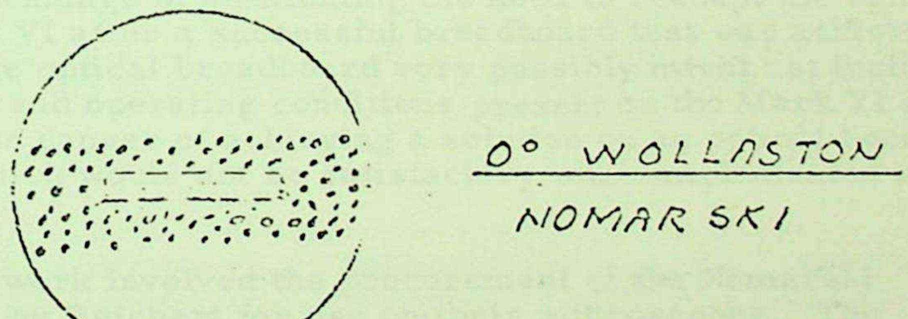
The lateral displacement approach has been successfully demonstrated (see Section 4 of this report) using a pair of Wollaston prisms with a third Wollaston prism used to restore parallelism



(a) Two Birefringent Lenses



(b) Two Wollaston Prisms



(c) Two Nomarski Prisms

Figure 9 Eyepiece Views of Final Image Using System Indicated

of the wavefronts. One drawback however, apparently arises from having both beams in focus in the specimen plane so that in the case in which both beams are passing through organic material, there will be no simple correlation between the instantaneous phases of the two beams which are passing through different particles at a given instant of time. Such a condition will occur probably in a majority of cases. Under this condition the lack of correlation between the phase shifts of the two beams will result in an essentially random waveform in the video output of the interference channel, containing little useful information aside from the indication of the presence of organic or other transparent material. For this reason, it is recommended that further work should be conducted to investigate the case of using this same approach with reduced beam separation, perhaps in the neighborhood of one micron or less. With adequately low separations between the two beams, it may be possible to obtain video waveforms which bear a relatively simple relationship to the cross-sectional shape of the particle as well as other basic parameters and thereby permit the application of basic pattern recognition methods to this type of system as has been done on stain-type PACT systems.

## 4.0 HARDWARE IMPLEMENTATION

The underlying objective of this program has been the development and implementation of an optical interference technique for the PACT optical scanner. The tasks of this program are discussed briefly in the Summary (Section 2) of this report and in more detail below.

Since all of these tasks relate to the hardware development on this program, they will be discussed in this section in terms of specific details of their execution (where applicable) on this program. In the following discussion, it should be noted that the tasks which are enumerated above are not purely a sequence of steps but that there are feedback loops through which a later task may produce a reiteration of an earlier task as, for example, has been the case between optical testing (Task 2) and optical design (Task 1).

### 4.1 Design of Optical System

The first task involved the initial design of the interference optical system. Included in this task are selection of suitable optical components such as the laser source, photodiode detectors, and birefringent optical components, and evaluation of certain items available on the market in terms of cost, availability, engineering parameters, etc. Also included in this task was a preliminary designing of hardware for mounting the optical components including the laser.

### 4.2 Conduct Optical Breadboard Tests

The second task originally was intended as a test of optical components on an optical assembly which was separate from the Mark VI system; however, a more direct approach was taken with even the preliminary breadboard work being performed on the Mark VI. This alternative had the advantage of eliminating the need to readapt the optical hardware to the Mark VI after a successful breadboard test was achieved. In addition, a separate optical breadboard very possibly might not include all of the components and operating conditions present on the Mark VI so that there would be the danger of achieving a solution on an optical bench type of breadboard which would not be satisfactory when implemented on the Mark VI.

Initial work involved the procurement of the Nomarski prisms manufactured by Reichert for use on their microscopes. The Nomarski prism approach was the first method to be investigated. Additional hardware procured included two rotatable analyzers, the 2 milliwatt helium-neon laser and the two silicon photodiodes for the interference and normal channels. In addition, the design was completed for the mounting hardware for the optical components, laser and photodiodes, and these components were installed on the Mark VI assembly, after the removal of the original photomultiplier tubes, zirconium lamp heaters and staining assembly. The removal of the staining assembly made it possible to move the impacter head closer to the optical scanner. This modification is of major importance in that it will reduce the dead time between the time that a challenge is picked up out of the air until it is sensed by the scanner, thereby reducing the critical time-to-alarm very substantially.

It should be noted that this task actually comprized a major proportion of the program and included not only the aforementioned subtasks but also additional work with other types of optical components. Specifically, this additional work involved the investigation of alternative schemes of implementing interference microscopy. The other schemes which were examined by means of hardware implementation are:

- Longitudinal displacement of foci of imaging and reference beams by means of thin birefringent plates cemented to condenser and objective lenses.
- Longitudinal displacement of foci by means of birefringent lenses.
- Lateral displacement of foci by means of a pair of standard Wollaston prisms.
- Lateral displacement of foci by means of a pair of standard Wollaston prisms, with a third Wollaston prism added in order to restore parallelism of the two beams after recombining.

These alternate approaches, of course, required the procurement of the specific additional optical hardware mentioned above, i.e. birefringent plates, birefringent lenses and Wollaston prisms, together with miscellaneous additional hardware and mounting fixtures.

Testing of the various optical configurations was conducted both visually and by means of waveform observations using an oscilloscope. For visual work, the image in the final focal plane of the interference channel was viewed through an eyepiece. During tests and alignment procedure, the two images of the scan (via the imaging and reference beams) could be viewed after the light had passed through a rotatable analyzer. Also, visual observation permitted examination of interference fringe patterns which resulted from mutual cancellation and reinforcement between the imaging and reference beams. Consequently, the observer could adjust the positioning of the optical elements to achieve a more nearly optimum interference pattern by observation of the interference bands. Also, an evaluation of the performance capabilities of the particular interference method being used was facilitated to a very large degree by this use of visual observation. On the other hand, the oscilloscope display of the video was useful in showing the degree of destructive interference between the two beams when viewed through the photodetector. This latter method provided greater sensitivity to variations in amplitude although it did not reveal as much information concerning overlap of the two images and geometry of the interference patterns.

## 4.3 Design Two Electronic Subsystems

The third task involved the design of two electronic subsystems to function with the Interference Optical Scanner. The first of these two subsystems in terms of function in the processing of the video outputs is the Two Channel Amplitude Comparator whose purpose is to discriminate between opaque and transparent particles on the basis of relative amplitudes in the normal and interference channel. An opaque particle such as a grain of dust, etc. is of no interest and thus should be rejected. Clearly an opaque particle will exhibit a large amplitude of both the normal (no interference) and the interference channel, whereas a transparent particle (such as an organism) will have a very low amplitude in the normal channel, but generally still will have a large amplitude in the interference channel.

This subsystem, as the name implies, accepts or rejects particles on the basis of the relative amplitude between the two channels. Thus, it is completely analogous to the red/green amplitude ratio detector circuits being used in the conventional system using staining, in which the amplitudes of the green and red channels are compared. Consequently the circuit developed previously for that application, i.e., the green/red ratio detector, was readily adaptable to the new application to the Interference Optical Scanner. Therefore, since the existing green/red ratio detector has a rejection threshold which is continuously variable, it was considered to be fully adaptable to this application as a Two Channel Amplitude Comparator with no internal wiring modifications. This subsystem originally was developed at El Monte under Contract DAAA15-72-C-0375. These two portions of the subsystem are wired on two Augat boards. For further information including diagrams and schematics on the circuits used, the reader is referred to the final report on the above contract.

The second electronic subsystem fabricated for this program is a Pulse Height/Pulse Width Discriminator. This circuit, also originally developed under the aforementioned contract, selects or rejects video signals on the basis of pulse height (amplitude) and pulse width. In order to not be rejected by this circuit, both of these two parameters must fall within certain ranges of values which are selected by potentiometer settings. Three such ranges, or "windows," are provided by the discriminator each of which is adjustable in position, height and width independently. This subsystem has five outputs, specifically the counts coming through each of the three windows, a fourth output giving the sum of all three and a fifth output providing counts through two of the three windows. These outputs may be fed into counters to monitor the numbers of counts coming through the system or they may be fed into other signal processing equipment. For more details regarding this circuit, the reader is referred to the final report for Contract No. DAAA15-72-C-0375.

## 4.4 Fabricate and Test the Electronic Subsystems

Subsequent to the planning of the electronic configuration the two subsystems described above were fabricated with two Augat circuit boards being used for the first subsystem (Two Channel Amplitude Comparator) and one board for the second (Pulse Height/Pulse Width

Discriminator). These circuits were checked out with bench tests and mounted in a special chassis assembly which was designed and fabricated for this purpose. (See Figures 10 and 11). In addition to the mounting connectors and cabling for these three circuit boards, this chassis includes the power supplies for their operation as well as the laser power supply, and controls for power, selection of windows on Pulse Height/Pulse Width Discriminator, and Threshold and Ratio settings for the Two-Channel Amplitude Comparator. Connectors are provided on the rear panel for the necessary interconnections (see Figure 12). In addition to the aforementioned electronics, amplifiers for the preamplifie outputs of the diode detectors were designed, fabricated and installed inside the two housings for the detectors. The basic schematic for these amplifiers remotely from the electronics chassis. These power leads enter the photodiode/amplifier housings through the same connectors as do the cables which carry the video output signals from amplifiers.

### 4.5 Modify the GFE System to Accommodate the New Optical Configuration

The optical subassembly of the GFE PACT system was modified in accordance with the optical changes required for the new interference system. Several changes were required and some of them have been mentioned above. Figures 14 and 15 show representative views of modifications in the optical subassembly. Figure 14 shows a rear view of the scanner, showing the position of the mounted laser, and the photodiode detectors mounted in place of the original photomultiplier tubes. The multi-pin male connectors for receiving power to the amplifiers inside the detector housing, and for video output from the amplifiers may be seen at left. The two micrometer knobs in Figure 14 are the knobs for rotating the two analyzers through which the two photodiodes view the incoming light. Figure 15 shows the front view of th modified scanner. Note that the staining and destaining devices have been removed and the impaction head now is located immediately before the roller next to the sprocket which precedes the optics. The meniscus applicator immediately precedes the impaction head. These modificatio result in a marked reduction in the time delay between impaction and observation which may be vital in that the system will be able to detect a challenge and sound an alarm before more harm has been done by the challenge organisms. Optical modifications may be seen at the top cente of the picture which was taken early in the program. Here a Nomarski prism may be seen in its slide-housing which is oriented vertically with its adjustment screw seen at top. Later modifications to the system greatly expanded the optical assembly in this location where the light emerges before entering the photodiodes. These modifications include a lengthening of the optical train to accommodate additional optical eleme and their positioning. This was accomplished by replacing the two slide rode with longer ones. Also additional fixtures were machined to suppo

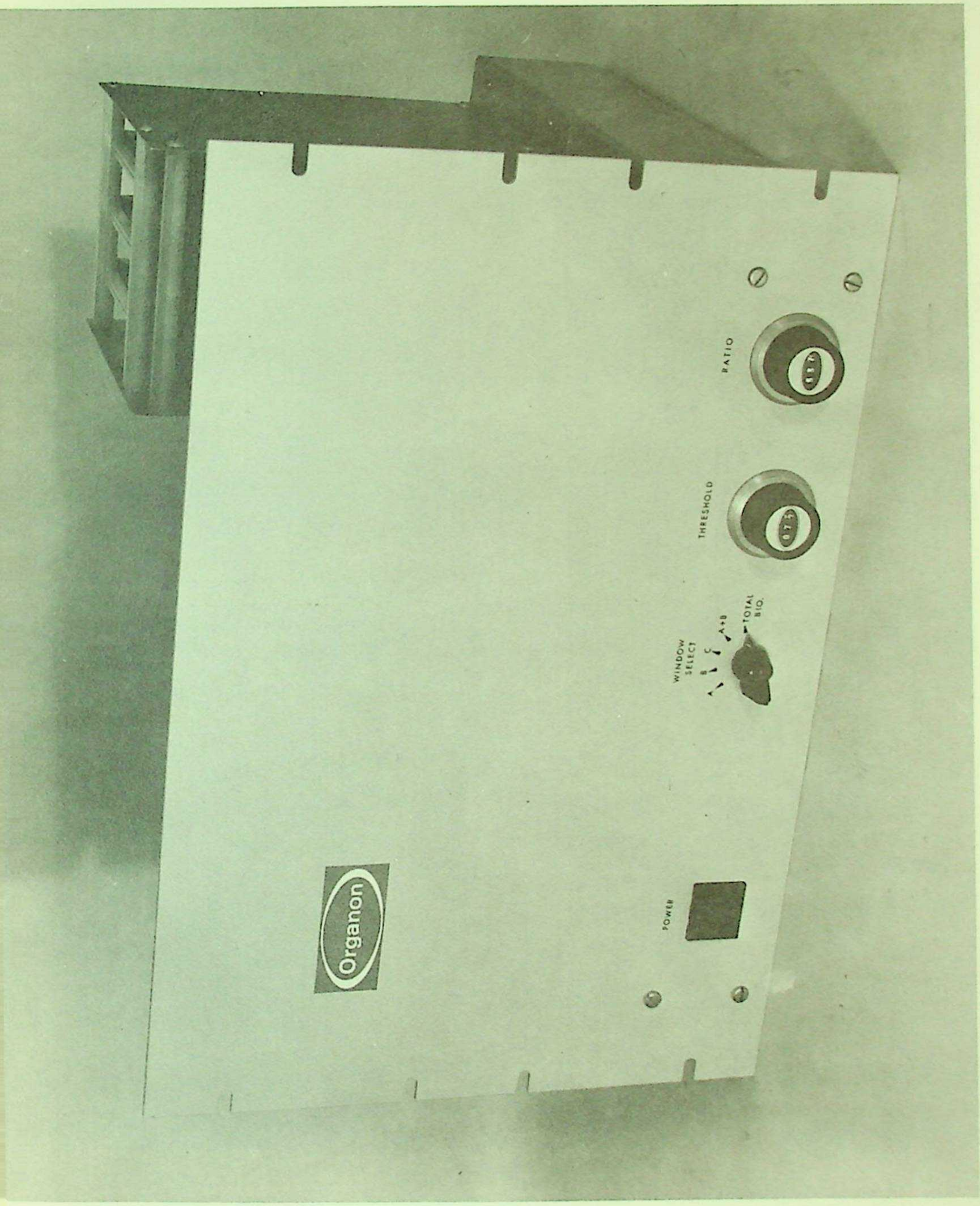


Figure 10 Electronic Subassembly (Front View)

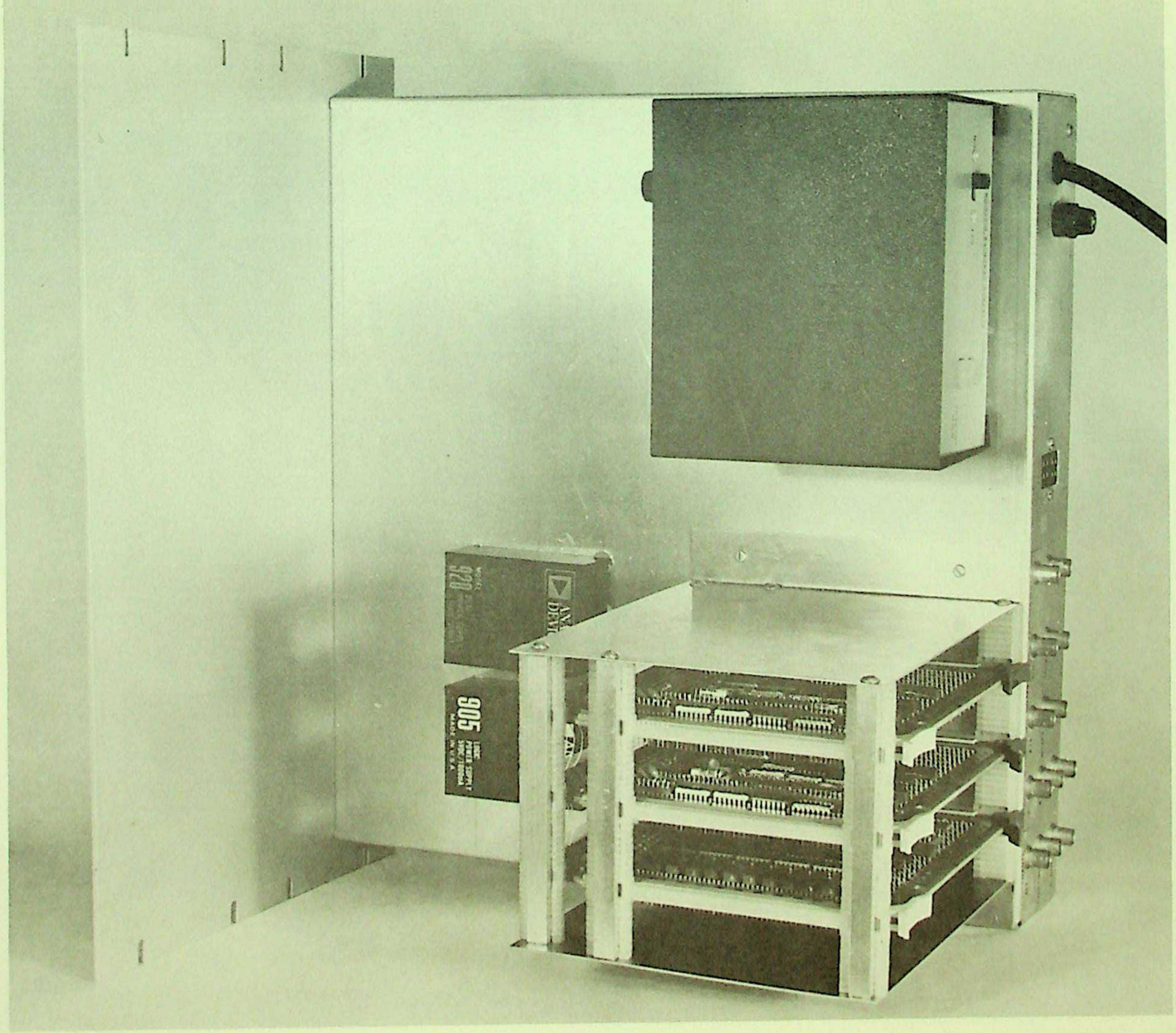


Figure 11 Electronic Subassembly (Top View)

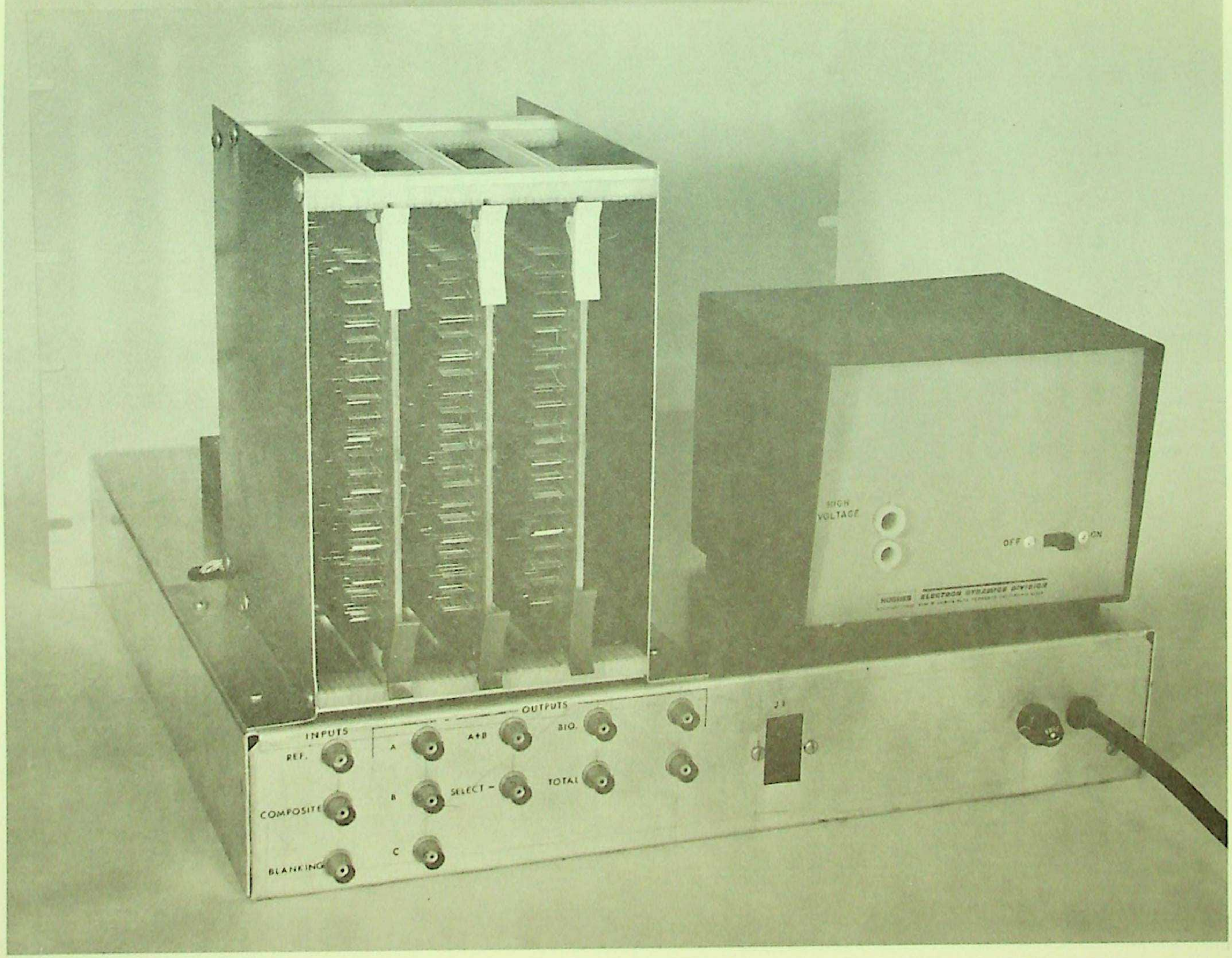


Figure 12 Electronic Subassembly Rear Panel

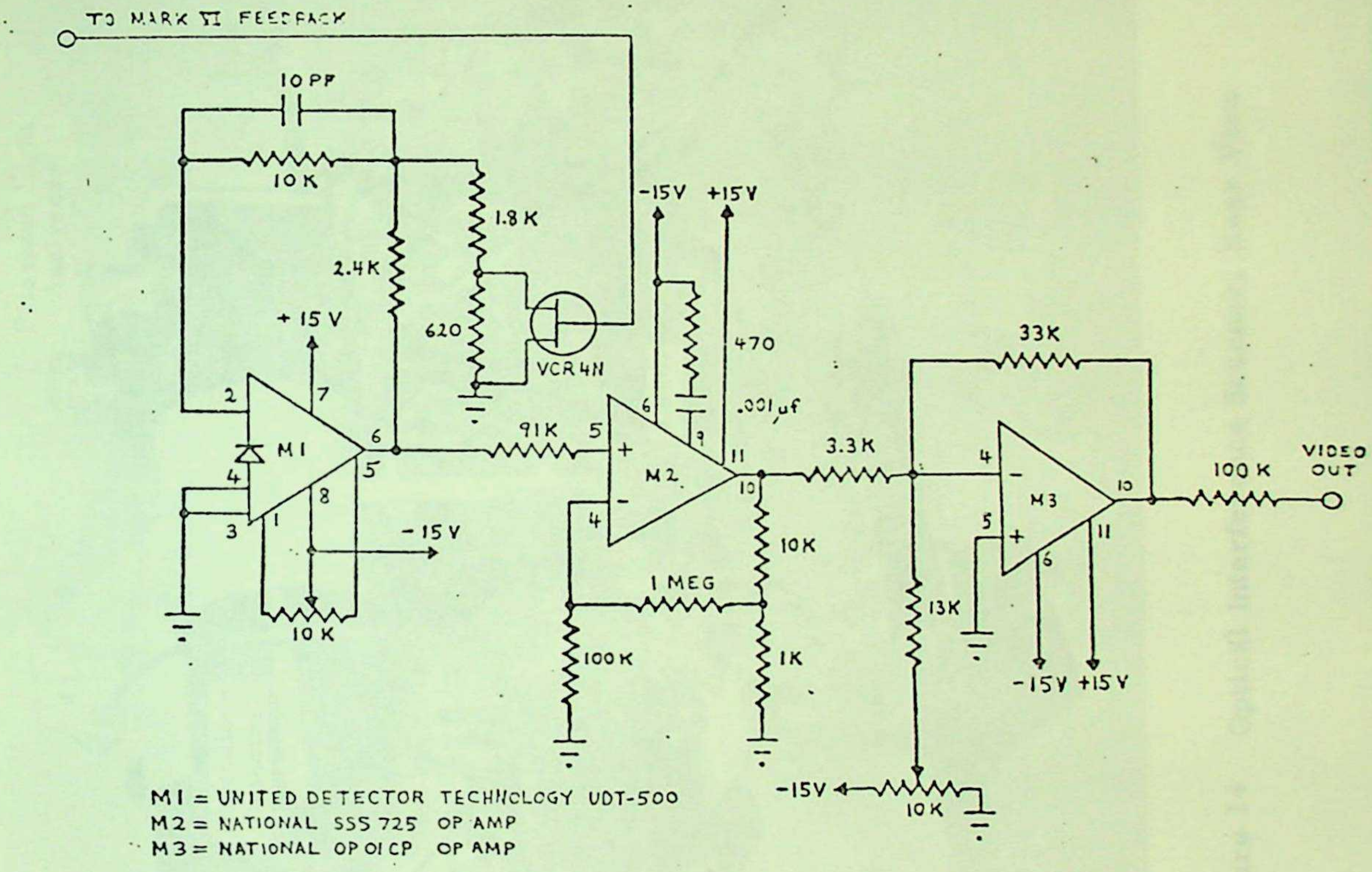


Figure 13 Video Amplifier Schematic

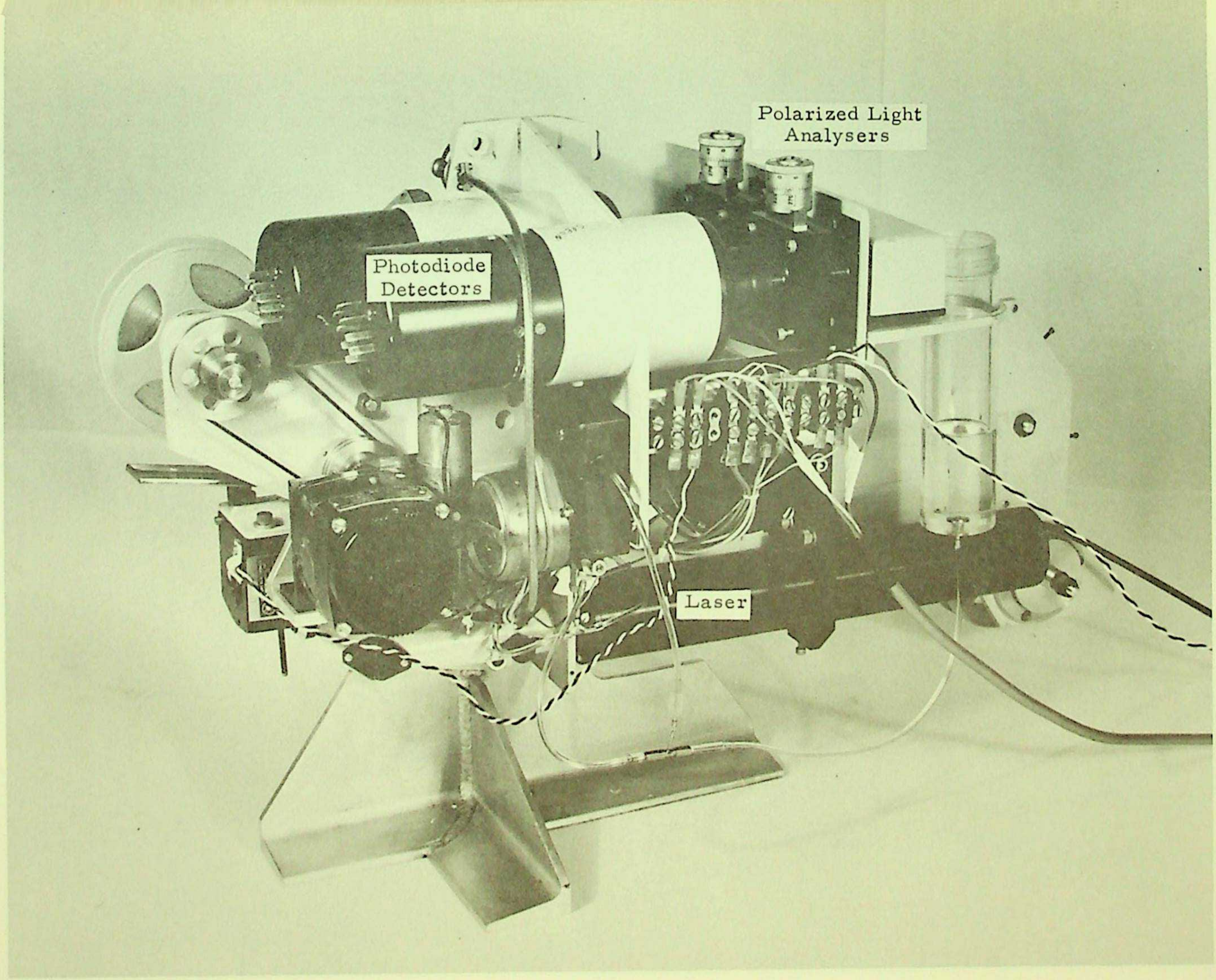


Figure 14 Optical Interference Scanner, Rear View

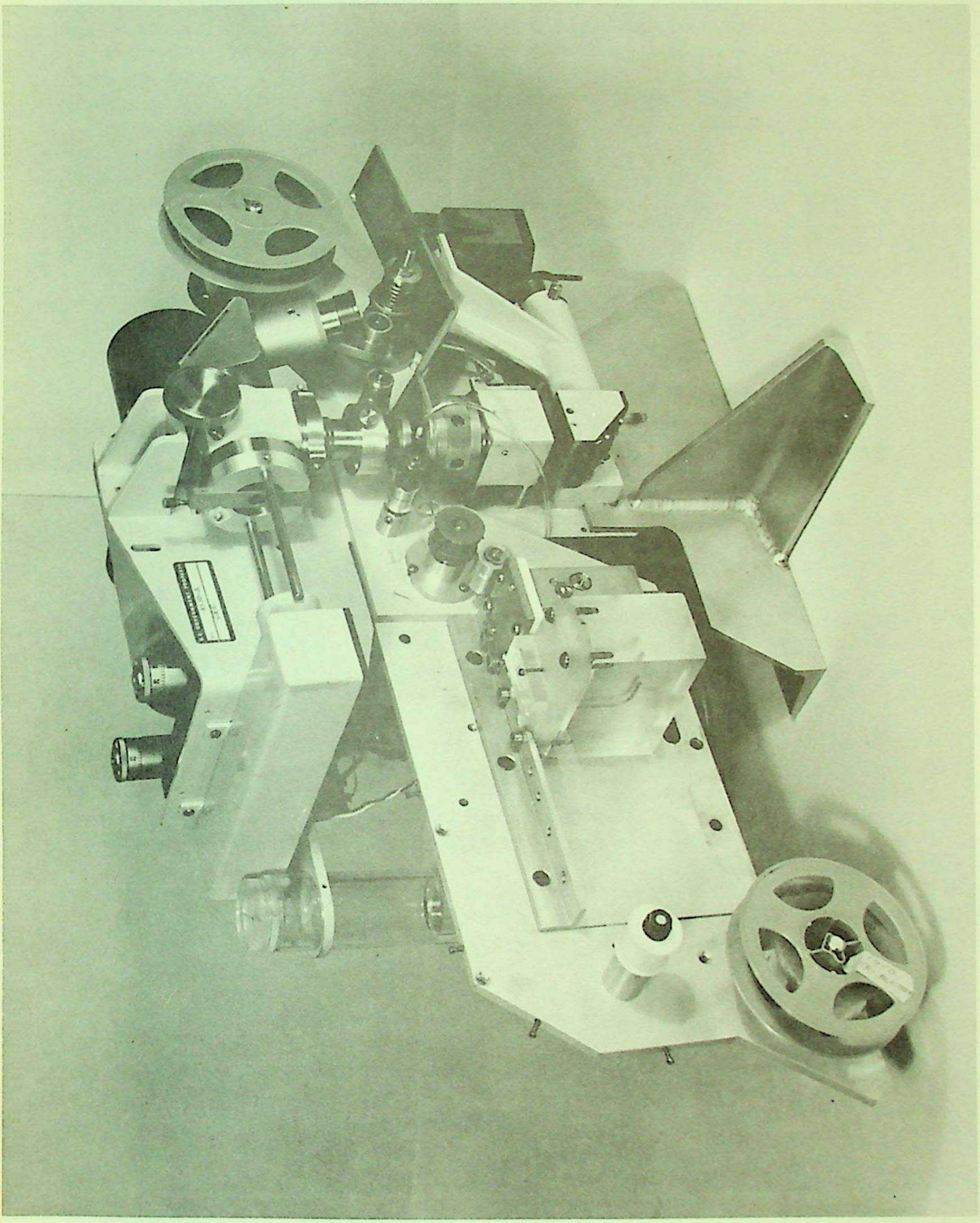


Figure 15  Optical Interference Scanner, Front View

other components as required. The eyepiece which was used for visual observation and analysis of the imaging was mounted in one of these fixtures after the removal of the rectangular housing which contains the mirrors etc. for relaying the light to the photodetectors. This housing is shown in its original position in Figure 15. In later work, which concentrated on the operation of the interference channel, one photodetector was removed from its normal mounted location and attached to an external support located behind the optical slide assembly, thus the rectangular reflector housing was not required in these breadboard tests. One of the rotating analyzers also was relocated on the optical slide assembly, having been remounted on a fashion similar to the Nomarski prism assembly shown in Figure 15. It should be pointed out that the arrangements just described were a type of breadboard and would be modified in an appropriate manner for use in a completed system. This breadboarding was necessitated by the extensive experimentation conducted during the program which had not been anticipated at the outset of the program. In a follow-on effort a permanent type of mounting should be provided. Figure 16 shows a close-up view of the optical assembly between the galvanometer (at lower right) and the tape (upper left). In the bottom center of the picture can be seen the modification to the tube between the spider and the diagonal mirror. This modification provides for positioning and adjustment of an optical element in this part of the light path. The modification, as seen in the figure, consists of a longitudinal slot in the side of the tube to permit positioning of the optical element along the optic axis and three vertical slits to permit its rotation at three locations. This modification was made initially for the application of the birefringent lenses (Rochon prism derivatives) in order to permit proper positioning of the lower lens. Later it was used also with the Wollaston prism as well. The holder of the optical element is partially visible in Figure 16 behind the knob used for its positioning. Figure 17 shows the optical slide assembly as it appeared at the time the birefringent lenses were installed in the system, one of which can be seen in the picture immediately to the left and below the property label. Protruding at the far left is an eyepiece used for visual examination at that time. Figure 18 shows a view of the same portion of the optical system as it appeared late in the program using the Wollaston prism approach in which one prism (before the condenser) was used to separate the beams, a second prism (after the objective) was used to recombine them, followed by a third which was used to restore parallelism of the two beams and hence also their wavefronts. The optical elements mounted in Figure 18 are identified (in direction of light travel from right to left) as follows:

1. Wollaston prism for recombining beams
2. Negative lens for convergence angle adjust (not shown)
3. Wollaston prism for restoring parallelism

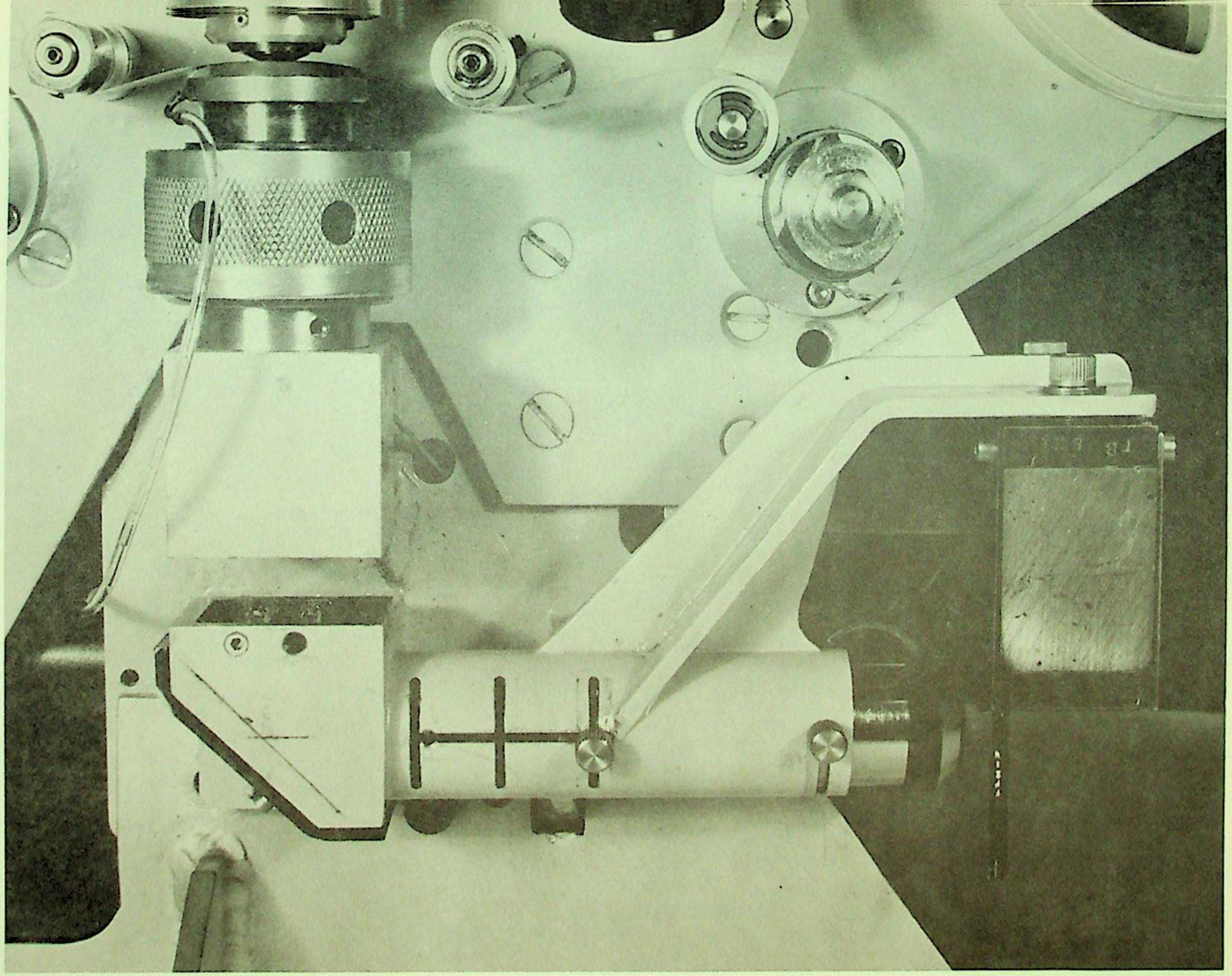


Figure 16 Modification of Mark VI to Accommodate Lower Prism

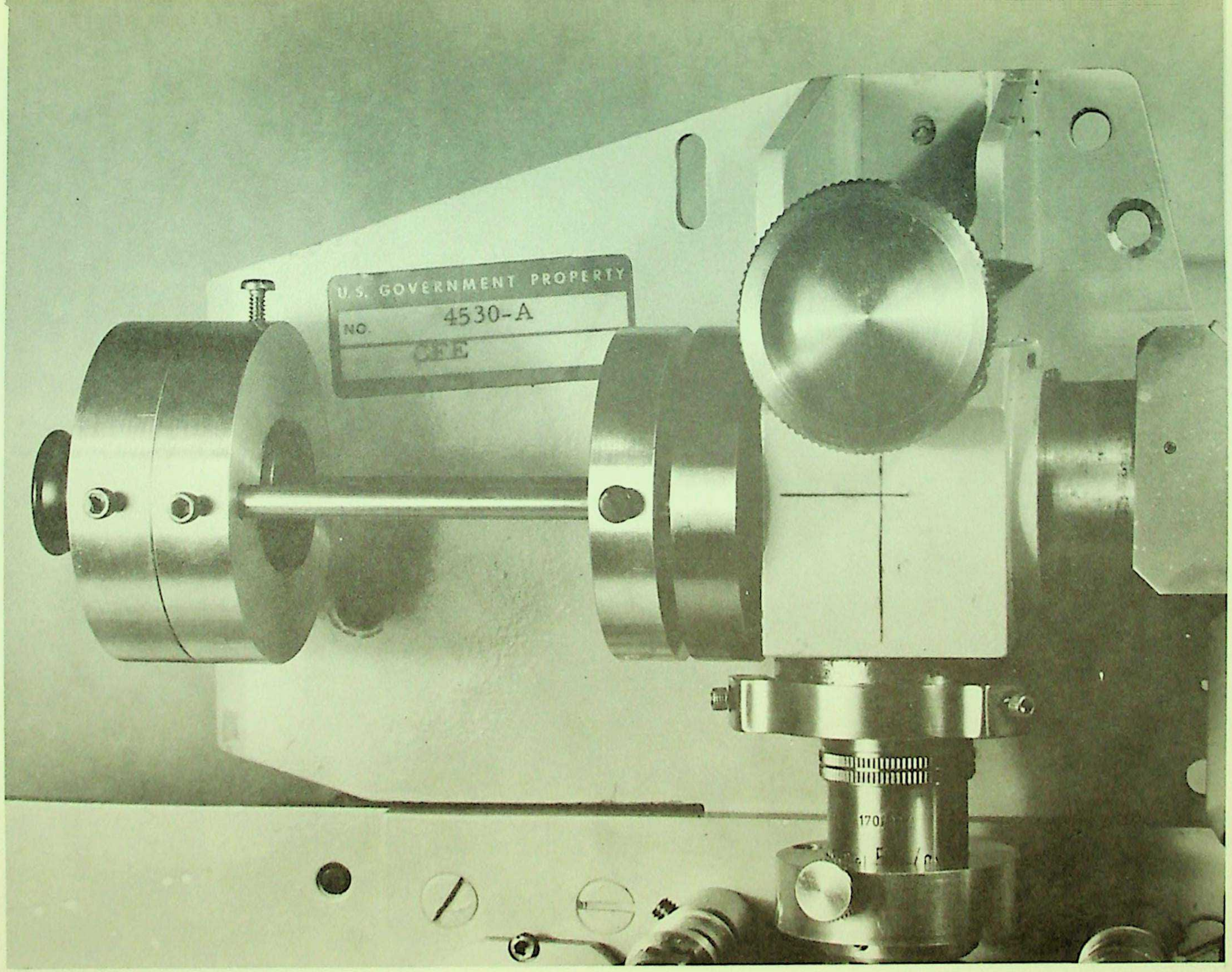


Figure 17 Optical Slide Assembly with Birefringent Lenses and Eyepiece

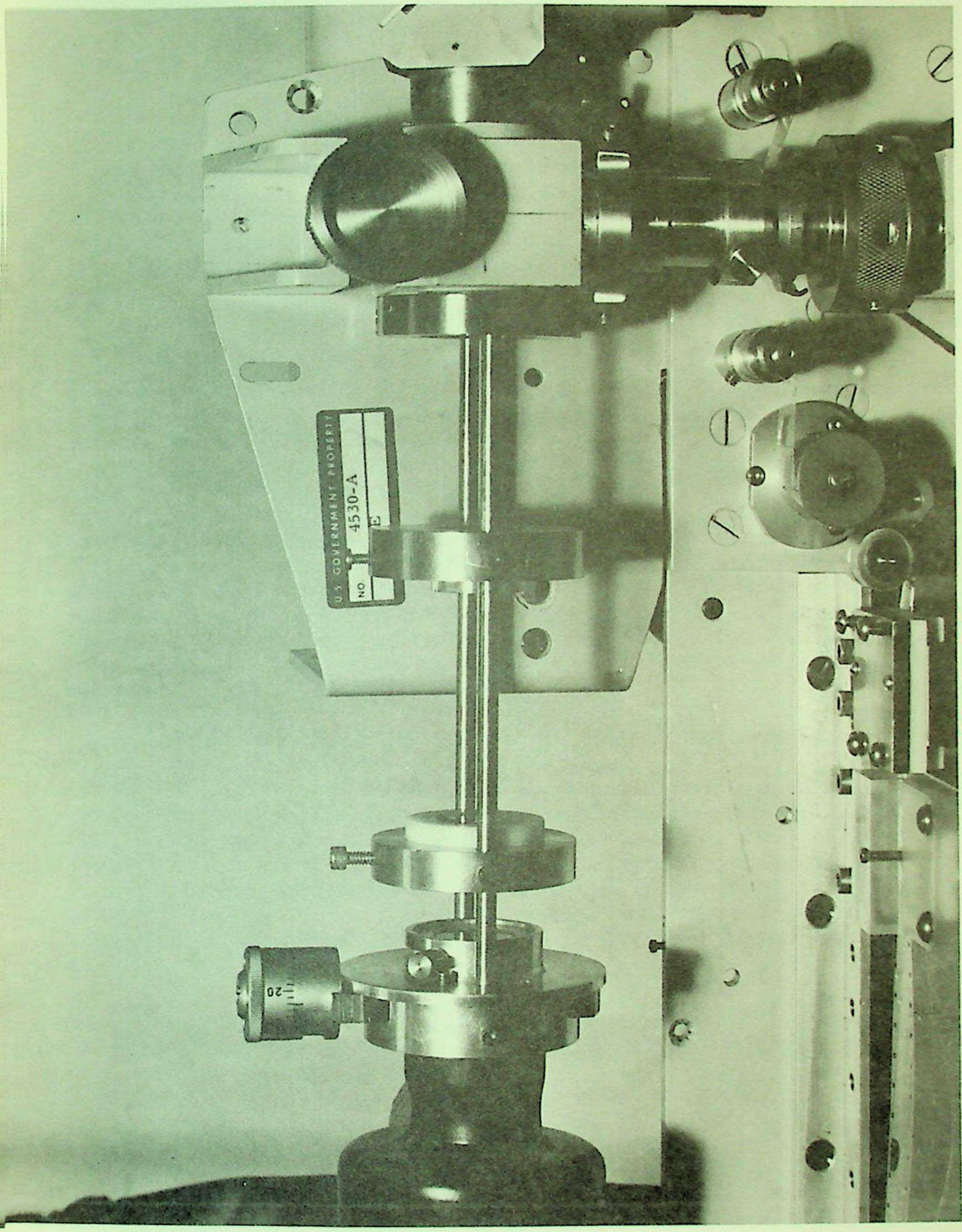


Figure 18 Optical Slide Assembly Using Three Wollaston Prism Approach

4. Assembly containing three components:

   a. Rotatable quarter wave plate

   b. Rotatable analyzer

   c. Eyepiece

A photodetector assembly also can be placed behind this group of components for displaying the video waveforms on an oscilloscope. It should be noted that provision is available for rotational adjustment of each component in the above list, although, of course, the negative lens and the eyepiece are not affected by rotation unless they are misaligned.

### 4.6 Integrate the Optical and Electronic Subsystems

The interfacing between the optical and electronic subsystems was limited to the operation of the photodetectors and monitoring their outputs on an oscilloscope. Numerous runs were made using carbon, mold spores, and wet Bg using different optical configurations. Due to difficulties in achieving phase interference on transparent organisms until the end of the program, it was not in the best interest of the program to perform additional interfacing between the electronic subsystems described earlier and the photodiode amplifier outputs. Even if such interfacing had been performed, no valid system testing and evaluation could have been made. This is an area which can be performed in a follow-on effort after perfecting the operation of the optical portion of the system. Once this primary goal has been achieved, interfacing and integration should be a very simple task performed in a few days.

### 4.7 Conduct a Final Checkout of the Integrated System

It is evident that the complete interfacing and integration of the optical and electronic subsystems must be completed before this task can be performed. This task would serve as a final checkout prior to shipment to reverify proper operation subsequent to more rigorous testing which would be performed on a variety of subjects including ACP (agent cloud particles) and uniparticulate subjects. The equipment finally would be shipped along with test data to the customer.

## 5.0 RESULTS AND CONCLUSIONS

The most critical part of this program has been the optical design since this forms the basis for the entire developmental program. The fundamental objective of the program has been to apply optical interference technology to the PACT system in order to eliminate the use of staining which is used in the present system. This change offers numerous advantages which have been discussed in the Introduction of this report. The greater part of the program was spent in devising, implementing and testing various approaches to interference microscopy as applied to the PACT system. A total of five different basic techniques was implemented and studied. Each of these systems separates the light into two orthogonally polarized beams whose foci are displaced from each other and subsequently recombines them. The basic concept of each system is to use the perturbations in one of the beams which is focused on the specimen of interest to alter the vector sum of the two beams in the focal plane and thereby produce a detectable change when examined through an analyzer. The theory involved here is discussed in greater detail in Section 3 of this report. The types of system examined may be grouped into two basic categories, according to the displacement of the foci of the two beams. These, as discussed earlier in the report, are lateral displacement systems and longitudinal displacement systems. The five categories examined fall into these two basic types of system as follows:

Lateral displacement:

- 2 Wollaston prism system
- 3 Wollaston prism system
- 2 Nomarski prism system

Longitudinal displacement:

- 2 birefringent plate system
- 2 birefringent lens systems

The longitudinal displacement systems have the advantage of having the reference beam out-of-focus in the specimen plane and thus it is less affected by objects crossing its path so that most of the interference action is caused by the object of interest rather than extraneous material. However, the longitudinal displacement system has proven to have some drawbacks; specifically, the two drawbacks which were discovered in this program related to one of the two approaches tried and the other drawback was found in the other. They are specifically as follows:

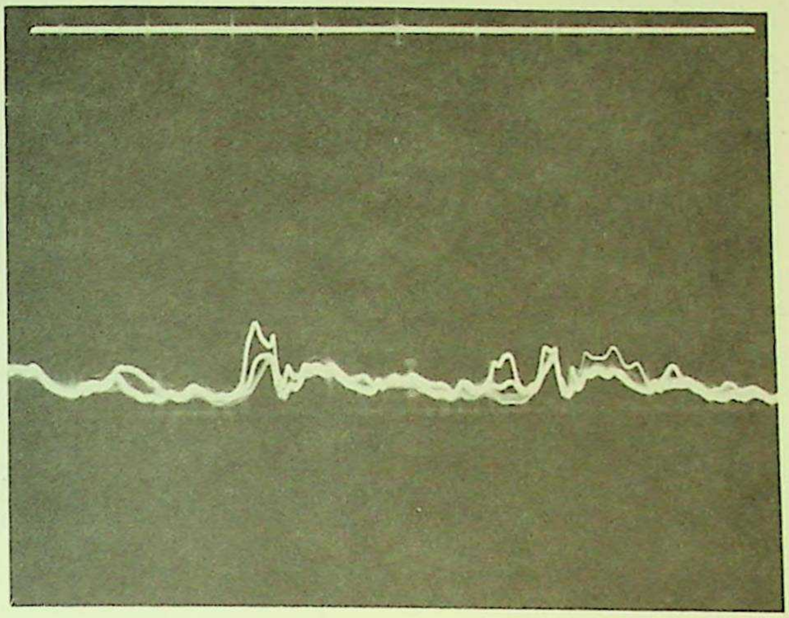
- It was found that the thin birefringent plates used between the lenses and the focal plane introduced enormous amounts of spherical aberration due to the high numerical aperture of the cones of light. Moreover, it was noted that the aberration effects of the two plates were additive rather than compensative. To correct this problem would require special condenser and objective lense designs which would compensate this aberration. Moreover, it is questionable whether this could be done successfully at such high numerical apertures. Thus, this approach was deemed to be impractical in this application. As discussed in Section 3, placement of the plates on the other side of the lenses is also an impractical approach because of the small beam separations which results.

- The system using two birefringent lenses, while it appears to give good freedom for aberration, also suffered from a non-compensatory geometrical effect similar to that described in the preceding case where the non-compensatory effect was in the foci of the various zones of light at the image plane resulting in severe spherical aberration. With the birefringent lenses the problem was one of different image magnifications. This in itself might not be a serious defect, since one image is produced by the reference beam which is used only for interference. An apparently associated effect which is a serious problem is the formation of circular interference fringes which were observed to have high spatical frequencies, and attempts to broaden them to an adequate degree were altogether unsuccessful. This approach, like the other longitudinal displacement approach mentioned above, does not appear to present a feasible solution to the problem at hand.

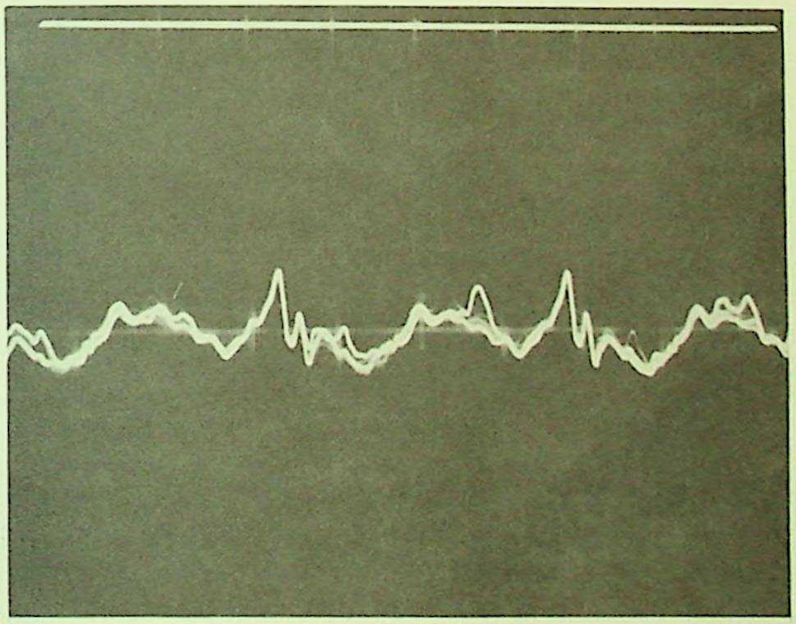
The lateral displacement systems, while having both beams in focus on the specimen plane, have appeared much more promising as solutions to implementing a practical and operational interference contrast system. Three basic approaches were tested on the Mark VI and the last two show considerable promise.

- The most basic system uses two Wollaston prisms; the first of these separates the two beams before the condenser, and the second recombines them after they have passed through the objective. This configuration may be regarded as a prototype of the two remaining systems which are discussed below, in that either of them represents a further development of this basic system. This first system however, suffers from the nonparallelism of the recombined light resulting in a fringing problem. These problems can be reduced greatly by either of the remaining approaches.

- The approach using three Wollaston prisms is a derivative of the system just described in which a third prism having the proper deviation angle is inserted after the second prism in order to restore parallelism of the emergent light. Results obtained with this approach, when used in the absence of immersion oil have been encouraging, (see Figure 19, 20 and 21) and high amplitude signals are obtained readily with unstained Bg. Figure 19 compares the video waveforms on the normal channel (no analyzer) and on the interference channel, using opaque carbon particles. Typically the normal channel has shown substantially larger amplitudes on opaque particles than the photograph in Figure 19 would suggest.

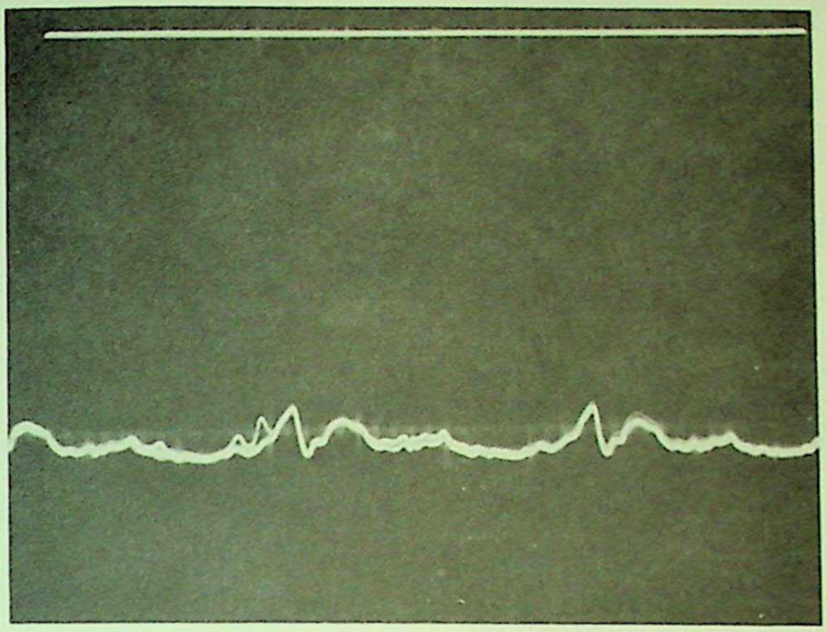


Reference Channel
No Analyzer

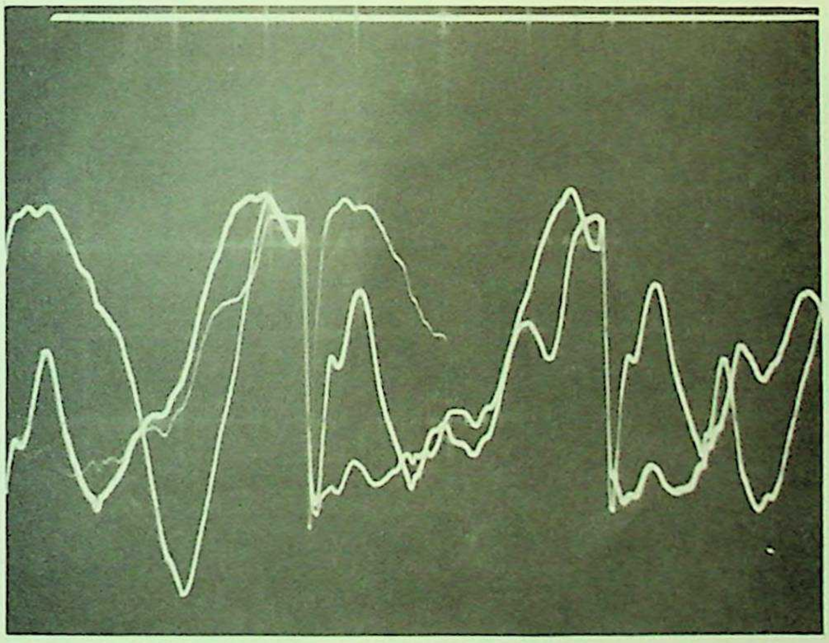


Interference Channel

Figure 19 Video Waveforms With Carbon

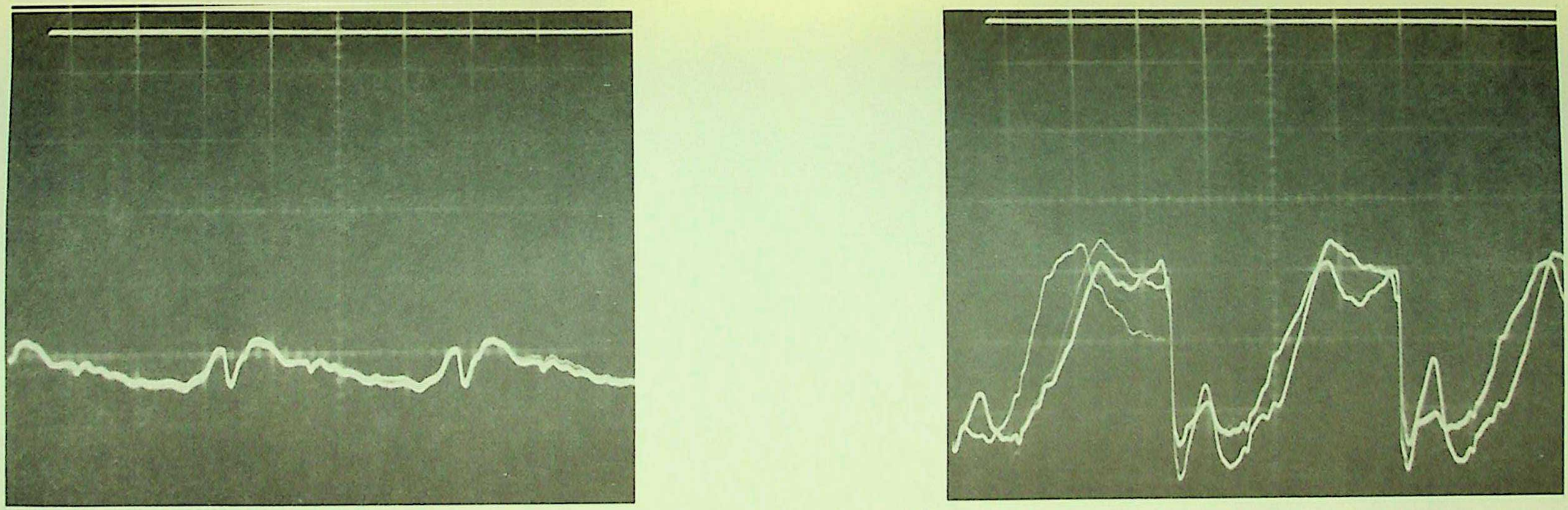


Reference Channel
No Analyzer



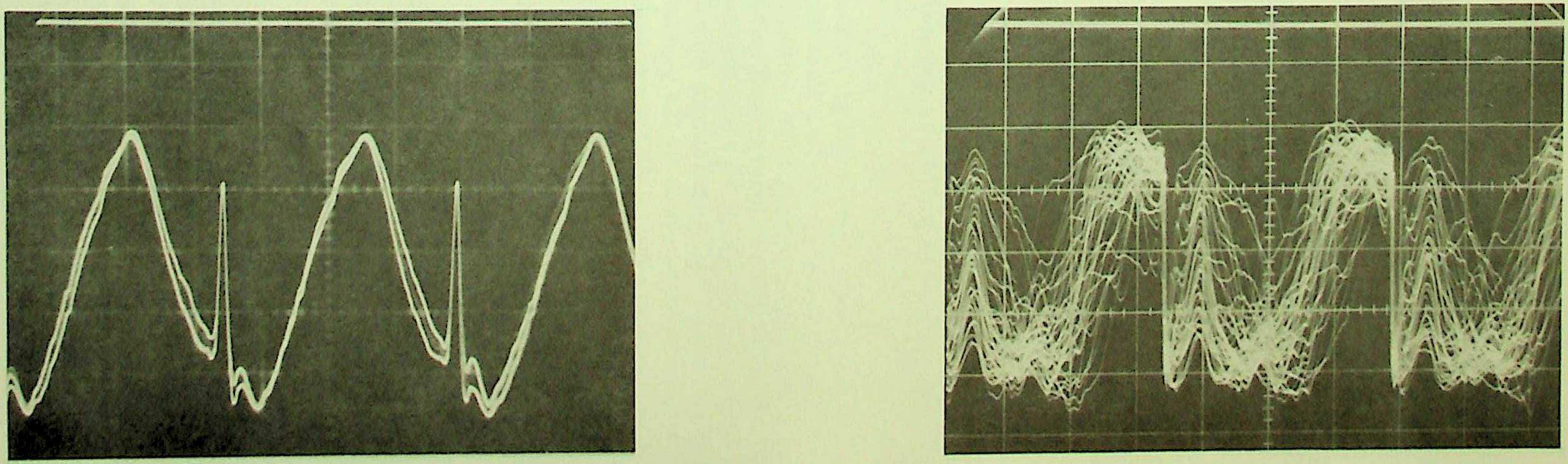
Interference Channel

Figure 20 Video Waveforms with Stained Bg



Reference Channel

Interference Channel - 1/100 Sec



Interference Channel - Clear Tape

Interference Channel - 1/10 Sec

Figure 21 Video Waveforms Comparing Reference Channel and Clear Tape with Unstained Bg

Figure 20 shows video waveforms obtained with this configuration using stained Bg. The zero line in the interference channel has a large curvature to it due to the nonuniform cancellation across the line; however, it is hoped that this can be worked out with further refining. The double trace in the interference channel is the result of two sweeps of the oscilloscope during the exposure, showing a change in video information. Figure 21 shows the results obtained with unstained Bg. Note that the reference channel amplitude (upper left) is low due to the transparency of the Bg. The interference channel with no agent on the tape shows a curved baseline (lower left) resulting from the incomplete cancellation problem mentioned earlier. The two exposures at right show the presence of unstained Bg. Note that the effect is essentially identical to that obtained with the same stained agent, (Figure 20) showing that staining is no longer necessary. The exposure at upper right shows two traces during the 1/100 second exposure (with vestiges of other traces probably due to phosphor persistence) again revealing the change in video data during successive traces. A 1/10-second exposure (lower right) shows many traces which are constantly changing as the Bg is moving across the trace.

It is evident that very encouraging results have been obtained, as shown in these photographs and prospects for further progress are also promising. One problem remaining is the complex structure of the video waveform caused by the Bg. Refinement of system parameters or going to the use of Nomarski prisms which use a much smaller beam separation hold promise for eliminating all or much of this waveform peculiarity. Tests using Nomarski prisms without oil also have yielded similar encouraging results to those pictured on unstained Bg, but without baseline curvature.

- The Nomarski system just mentioned is basically the same as the 2 Wollaston prism approach except that it uses a pair of Nomarski prisms (see Section 3). No third prism is used; nevertheless, the interference bands are quite broad. It is believed that this approach may be highly successful when implemented without oil and tests indicate that it substantially solves the curved baseline problem. Also, the complex structure of the video waveform as obtained with the 3 Wollaston approach appears greatly diminished.

One very important consideration is the question of whether to use oil immersion in the interference optical scanner, as has been used in the past. Immersion oil was used during most of this current program. However, it should be pointed out that the index of refraction of immersion oil may be too close to the index for transparent organisms in most cases to make the latter visible by interference contrast. This is because the operation of interference contrast is based upon the reduced phase velocity of propagation through the object of interest. If, however, the surrounding medium has almost the same index and hence phase propagation velocity, little or no phase shift between the imaging and reference beams will be observed; thus the interference contrast will be defeated. It is doubtful if the available range of indices of refraction of available immersion oils is sufficient to circumvent the problem. Thus, it is urged that the immersion oil be deleted and air optics used in the interference contrast system. The loss in resolution at the output of the video that would result from the reduced numerical apertures inherent in dry objectives should not be detectable if good optics are used, since the 40 X objective lens already in current systems is being used dry, thereby limiting the numerical aperture.

## 6.0 RECOMMENDATIONS

The aforegoing discussion has summarized the progress in the optical work during this program. Due to the larger portion of the effort being devoted to this task than originally was planned, it was not possible to do the final integration and checkout of the system. Considerable progress was made in the research and development effort toward the goal of developing the Interference Optical Scanner, and the remaining integration and testing should be performed quickly and easily once the optical interference operation has been perfected.

The present program has helped to demonstrate the feasibility of the PACT Interference Optical Scanner. The purposed future work would concentrate on refinement of the optical technique and result in an operating prototype PACT Interference Optical Scanner integrated with the electronics which has been fabricated and bench tested under the current program. This entire system would be checked out, tested on various agents and shipped to the customer.

The proposed effort is comprised of the following tasks:

- Design and fabricate a new optical subassembly compatible with an interference optical approach. Developmental work under the present contract has been severely hampered by deficiencies in the present Mark VI optical subassembly. These deficiencies are the following:
    1. Excessive use of folds and bends in the light path resulting in:
        a. Alignment difficulties
        b. Light scatter due to the excessive number of optical surfaces as well as misalignment
        c. Excessive number of optical components making proper placement of interference components difficult or impossible.
    2. Lack of rigidity of the mechanical mainframe, resulting in severe vibration problems as well as difficulties maintaining proper alignment.
- Refine and finalize the optical technique. This task involves the refinement and finalization of the lateral displacement interference optics with the refinement having a two-fold objective:
    1. Achieve an acceptably uniform null across the scan to obtain a straight baseline to the video waveform across the scan.

2. Adjust parameters to achieve a video waveform structure which is representative of the cross-sectional structure of the material being scanned.

Subsequent to this refinement subtask the optical subsystem would be finalized as an operating prototype.

- Integrate the optical and electronic subsystems. This task includes the interconnection of the video outputs from the photodiodes and their preamplifiers with the electronic subassembly built on the previous program. The entire system then will be tested with uniparticulate and ACP simulants: Bg, Sm and egg, as well as with background particles. The resultant data then will be analyzed and the system adjusted or modified to achieve optimum results.
- The complete system will be given a final checkout to insure that it is operating properly and the complete system then will be shipped along with analysis and test data to the customer.

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